MANAGING ROOT-KNOT NEMATODES IN GREENHOUSE AND MICROPLOT EXPERIMENTS WITH ORGANIC AMENDMENTS

By

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Eight different organic amendments were utilized to suppress root-knot nematodes (*Meloidogyne incognita* and *M. arenaria*) in several experiments. In the greenhouse, inoculation of two-week-old seedlings of 'Clemson Spineless' okra (*Hibiscus esculentus*) provided the best conditions for building up root-knot nematode populations and studying populations dynamics. Plant growth and nematode suppression were higher when amendment was placed on soil surface rather than incorporated into soil. In another study, castor (*Ricinus communis*) and velvetbean (*Mucuna deeringiana*) soil amendments resulted in better plant growth and nematode suppression than collard

(*Brassica oleracea*), zinnia (*Zinnia elegans*), sesame (*Sesamum indicum*), and 'SX-17' sorghum-sudangrass (*Sorghum bicolor* x *S. sudanense*). For velvetbean and castor amendments, a rate of 5 g per 560 cm^3 of *M. arenaria* infested soil was best for plant growth and nematode suppression. Irrigation frequency, time for amendment decomposition, and inoculum density were also important factors affecting plant response. However, it cannot be generalized that amendments with low C/N ratios will always suppress nematodes and improve plant growth if conditions of soil moisture, temperature, plant host, and nematode species are not kept constant.

A three-year microplot experiment with perennial peanut (*Arachis glabrata*) hay and yard-waste compost (mainly woodchips) revealed greater treatment responses with peanut hay at low or high nematode (*M. arenaria*) levels, probably because this amendment had a lower C/N ratio. Root-knot nematodes impaired nutrient uptake, thus high residual levels of nutrients were registered in soil under the high nematode treatment. Nutrient analysis within okra plants varied among plant tissue and plant growth stage. Residual levels of nutrients in soil affected growth of a subsequent rye (*Secale cereale*) cover crop, and high levels of root-knot nematodes affected dynamics of the stubby-root nematode (*Paratrichodorus minor*).

Plant growth and nematode suppression were demonstrated with use of organic amendments, but an integrated management approach, by using an organic amendment with low C/N ratio and a non-host crop, could be adopted for more effective use of the amendment in suppressing nematodes and improving plant growth.

CHAPTER 1
INTRODUCTION

Background and Previous Work

The effects of organic amendments on soil microbiological population dynamics have been reported since early in this century. The first scientific research with the use of organic matter to control plant-parasitic nematodes is attributed to Linford et al. (1938). Since then, the interest in using organic amendments to reduce nematode populations has increased (Mankau and Minteer, 1962; Miller et al., 1973; Sitaramaiah and Singh, 1978; Muller and Gooch, 1982; Rodríguez-Kábana, 1986; Rodríguez-Kábana et al., 1989; Vicente and Acosta, 1987; Gallaher and McSorley, 1994; McSorley and Gallaher, 1995a).

A number of organic amendments have been used for management of nematodes, because they are associated with reduced infection or survival of the nematode species, or because they may increase microbial and animal species antagonistic to nematodes (Linford et al., 1938; Watson, 1945; Mankau and Minteer, 1962; Mankau, 1968; Sitaramaiah and Singh, 1978; Rodríguez-Kábana, 1986; Brown, 1987; Rich et al., 1989). For instance, some of the diverse soil amendments used in the management of plant-parasitic nematode include chopped leaves of pineapple (*Ananas communis*) (Linford et al., 1938); cellulose and sawdust (Miller and Edgington, 1962); peat moss (O'Bannon, 1968); paper, cotton (*Gossypium hirsutum*) seed meal (Miller et al., 1968); dry crop residues, lespedeza hay, or oat straw (Johnson et al., 1967); oil

cakes, castor (*Ricinus communis*), mahuva, margosa, sesame (*Sesamum indicum*), peanut, mustard, neem, cotton seed, linseed, farm yard compost, chicken manure, alfalfa, oat hay, lespedeza (Rodríguez-Kábana, 1986); yard waste compost (McSorley and Gallaher, 1992); and also *Pongonia glabra*, *Crotalaria juncea*, *Azadirachta indica*, *Melia azadirachta*, and *Sebania aculeata* (Singh and Sitaramaiah, 1966). Nonetheless, the use of the organic amendments to manage plant-parasitic nematodes has not been fully accepted or understood as a biological control method because of the many interactions with environmental factors (Linford et al., 1938; Watson, 1945; Mankau, 1968). Some reports associate organic amendments with reduction of gall formation, reduction of hatched juveniles, or delay in egg hatching (Morgan and Collins, 1964; Johnson et al., 1967; Mankau. 1968; Singh and Sitaramaiah, 1994), while others show improvement of plant health, tolerance to nematode infection, or even inconsistent results (Ichinohe, 1985; McSorley and Gallaher, 1995a). In one study, reduction of juvenile numbers was reported, but no toxic substances were detected (Mankau and Minter, 1962); in another case, the number of juveniles remained the same, but infection and survival were reduced (Mankau, 1968).

The mechanism of action of organic amendments in improving plant growth has been attributed to the improvement of soil structure and aggregation resulting in increased aeration and water-holding capacity, to improvement in plant nutrition, or to the enhancement of the growth of organisms able to compete with or destroy nematodes (Stirling, 1991). Some results from the use of the organic amendments are attributed to increase in soil fertility, while others are attributed to toxic substances released from amendment decomposition (Norton, 1978; Rodríguez-Kábana, 1986; Rich et al, 1989;

Singh and Sitaramaiah, 1994). Effects reported may have resulted from the release of phenolic compounds, NH_3 or nitrite, Ca^+ ions, from changes in soil pH or soil moisture, or from the type, amount, and C/N ratio of the organic amendment (Holtz and Vandecaveye, 1938; Watson, 1945; Mankau and Minteer, 1962; Singh and Sitaramaiah, 1967, Mankau, 1968; Walker, 1971; Rodríguez-Kábana, 1986; Brown, 1987). Thus, the efficacy of amendments is highly variable, which needs to be considered when interpreting results.

Some problems in the evaluation of the efficiency of an organic amendment may be due to the difficulty in separating the use of the amendment as a nematicide or as fertilizer (Muller and Gooch, 1982). In addition, stress on crop growth, such as low soil moisture or fertility, might unbalance the system, causing fluctuations in nematode population levels (Nusbaum and Ferris, 1973). Furthermore, contradictions in research results may be due to lack of complete information on mineral analysis of the amendment, variation in the rates of amendments used, susceptibility of the crop plant as an indicator of nematode infection, monitoring of experimental conditions, or different nematode species. Of eight organic amendments tested by Mankau and Minter (1962), only steer manure failed to reduce *Tylenchulus semipenetrans* and castor pomace eliminated all citrus nematode larval stages but did not produce a substance toxic to the nematode. Nonetheless, in another study, incorporation of green manures into soil was effective in reduction of nematode infection (Johnson et al., 1967). For instance, plants growing in chitin-amended soil containing root-knot nematodes were larger and the roots were less necrotic than plants growing in nonamended soil, even though the degrees of galling were similar (Bergeson et al., 1970). Alfalfa- or soybean-amended soils inhibited

egg hatching, and as rates of these amendments were increased, more hatching inhibition occurred (Johnson and Shamiyeh, 1975).

Whatever the mechanisms involved are, the quality and quantity of the organic amendment used are important, but comparisons between reports are difficult since effectiveness of the amendment is highly dependent on the C/N ratio, crop susceptibility, nematode species, and environmental conditions. In addition, nematode-infected plants often exhibit symptoms of nutrient deficiencies because nematode infection impairs nutrient uptake (Trivedi and Barker, 1986). Furthermore, cultural practices may result in inconsistent reductions of nematode populations, which results in variable plant performance since yield of susceptible plants depends on the nematode species and density (Seinhorst, 1965, 1966, 1967). Thus, experiments involving organic amendments for nematode suppression should maintain adequate conditions for plant growth so that results could be better interpreted.

Another problem to be considered is that results obtained from greenhouse experiments or from laboratory tests may differ from results obtained under field conditions. For instance, Johnson et al. (1967) reported that oat straw amendment had reduced root-knot galling by an average of 75% in greenhouse experiments; however, under field conditions, effectiveness of the amendment was reduced.

The quantity and quality of the amendment used have a critical effect on the results. For instance, high rates of yard-waste compost improved crop tolerance to nematodes (McSorley and Gallaher, 1995a). Nonetheless, these authors mentioned that effectiveness of this amendment depended, among other factors, on supply and quality, and availability of the amendment in the field. In another study, Johnson et al. (1967)

found that although high rates of the oat straw provided better results reducing root-knot severity, maintenance of the soil amendment for a longer duration allowed for more decomposition and gave better results than when the amendment was maintained for a shorter duration. On the other hand, Linford et al. (1938) reported that 300 g of soil amendment were more effective than 400 g per 2,400 g of soil. Nusbaum and Ferris (1973) emphasized that decomposition rate of an amendment depends on the soil type and the climate. Variation in amendment responses also occurs if the amendment is used as seed, or as a cover crop, or if substances extracted from the amendment are used (Johnson et al., 1967). Furthermore, Rich and Rahi (1995) observed that leaves, rather than other plant parts of the soil amendments (castor, crotalaria, hairy indigo, and wheat) were more effective in limiting egg production of root-knot nematodes.

Much research on the use of organic amendments is still needed before growers could apply organic amendments as an alternative practice for managing plant-parasitic nematodes. Nonetheless, there is still a need of finding safe and economical alternatives to manage plant-parasitic nematodes because of the high cost and reliability of chemical nematicides (Trivedi and Barker, 1986). However, the dynamics of the nematode population under organic amendments may not be related to better yields, since the organic amendment itself may promote healthy plants, as well as improvement in soil moisture. Thus, some crops may better tolerate the presence of nematodes, without showing decreased yields (McSorley and Gallaher, 1995a).

Recently, an attempt has been made to solve a waste disposal problem through the recycling and composting of municipal wastes (Esser and Gaskalla, 1994). The positive effects of amendments on plant growth may provide incentives to recycle many different

types of organic materials in agricultural systems and to improve research on the use of amendments for management of plant-parasitic nematodes.

Research Objectives

Some available organic amendments were chosen for greenhouses studies and field experiments, because in the literature, they were reported to suppress nematodes when used in crop rotation, incorporated into soil, or applied as mulch (Watson, 1922, 1936, 1945; Watson and Goff, 1937; Lear, 1959; Mankau and Minteer, 1962; Mankau, 1968; Mian and Rodríguez-Kábana, 1982; Huang, 1984; Rodríguez-Kábana, 1986; Rodríguez-Kábana et al., 1988; Rich et al., 1989; McSorley and Gallaher, 1993; McSorley et al., 1994; McSorley and Dickson, 1995).

The main objective of this research, conducted under greenhouse conditions, was to compare the effect of the organic amendment on nematode population levels. For all experiments, plant growth parameters were measured on the root-knot susceptible 'Clemson Spineless' okra (*Hibiscus esculentus* L.). Specific objectives of greenhouse experiments were a) to compare placement of a yard-waste compost amendment (mainly woodchip) on different concentrations of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood race 1; b) to determine the effects of different inoculum concentrations of the root-knot nematode and different planting dates of okra on nematode populations and on okra growth; c) to measure the effects of irrigation frequency under different amendments on okra growth; d) to determine the effect of fresh and dry material of castor (*Ricinus communis* L.), collard (*Brassica oleracea* L.),

sesame (*Sesamum indicum* L.), 'SX-17' sorghum-sudangrass (*Sorghum bicolor* [L.] Moench x *S. sudanense* [Staptf] Hitchc.), zinnia (*Zinnia elegans* L.), and velvetbean (*Mucuna deeringiana* [Bort.] Merr.) organic amendments on okra growth and numbers of root-knot nematodes (*Meloidogyne arenaria* (Neal) Chitwood race 1). Authorities disagree over the scientific name of velvetbean, recognized as *Mucuna deeringiana* by some authors (Duke, 1981). Others consider it a form of the polymorphic species *M. pruriens* (Bogdan, 1977); e) to compare the effects of the two amendments (velvetbean, castor) which were most effective under the conditions previous studied; and f) to determine the effects of various rates of these amendments (velvetbean, castor) on nematode suppression.

A field experiment was conducted in microplots naturally infested with high and low levels of *Meloidogyne arenaria* race 1. The objective was to compare the effect of two amendments with different C/N ratios on nematode population densities over time under two different initial levels of the nematode. Specific objectives were a) to compare effects of peanut hay and yard-waste compost (mainly woodchip) amendments on root-knot nematode dynamics and growth of 'Clemson Spineless' okra; b) to compare soil mineral nutrient concentrations under the two nematode levels and different soil amendments.

CHAPTER 2
EFFECT OF ORGANIC AMENDMENT PLACEMENT AND INOCULUM DENSITY OF *MELOIDOGYNE INCOGNITA* ON OKRA SEEDLINGS

Introduction

Application of organic amendments has frequently been attempted as a means of managing plant-parasitic nematodes. Nonetheless, the mechanism of organic amendment activity against nematodes is not clear (Muller and Gooch, 1982; Duncan, 1991). Some possible explanations are that the organic amendment may promote good conditions for plant growth, may provide a favorable environment for the development of organisms antagonistic to plant-parasitic nematodes, or may release toxic concentrations of ammonia and phenolic compounds which may kill plant-parasitic nematodes (Mankau, 1968; Muller and Gooch, 1982; Trivedi and Barker, 1986; Duncan, 1991). Whether compounds such as ammonia released upon organic amendment degradation are nematicidal depends on the amount released, the C/N ratio of the amendment, and environmental conditions, so that results may be highly variable (Rodríguez-Kábana, 1986; Stirling, 1991). Organic materials with low C/N ratios are decomposed rapidly by microbes that readily attack water-soluble substances, hemicelluloses, and cellulose. Woody plant materials contain lignin, which is low in plant nutrients and is quite resistant to microbial attack. Unless extra N is provided, woody plant material decomposes slowly. Nonetheless, woody residues make excellent mulches for blueberries (*Vaccinium* spp.), fruit trees, ornamentals, and garden crops (Reuszer, 1957). Organic mulches may

enable plants to better tolerate nematode damage by increasing soil water holding capacity (McSorley and Gallaher, 1995a).

The root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood is particularly damaging to many vegetable crops such as okra (*Hibiscus esculentus* L.) (Christie, 1959). Reduction in survival and infectivity of *M. incognita* was obtained in fields treated with organic amendments (Mankau, 1968). Gallaher and McSorley (1994) observed some, but inconsistent, reductions of nematode populations by using yard-waste compost (mainly woodchips) as mulch. In contrast, Ichinohe (1985) reported improved yield of black pepper (*Piper nigrum* L.) by use of mulch, but did not observe reductions in the number of nematodes. The objectives of this study were to compare the effect of organic amendment placement (on soil surface, mixed in soil, or none) on three different concentrations of root-knot nematodes and on growth of okra under greenhouse conditions.

Material and Methods

The experiment was carried out in a greenhouse in the spring of 1994. Plastic pots 10 cm in diameter and 9 cm high were filled with 700 cm^3 (740 g dry weight) of steam-sterilized field soil mixed with sand, in a ratio of 1:1 by volume. The composition of the mixture was 92% sand, 4% clay, and 4% silt. On 14 February 1994, two-week-old seedlings of the okra cultivar 'Clemson Spineless' were transplanted into the pots after applying 0.6 g of granular 6-6-6 (N-P-K) fertilizer. The pots were watered immediately after transplanting. Plants were sprayed two times a week with dilute soap solution to reduce infestation of whiteflies (*Bemisia tabaci*).

The experimental design was a 3 x 3 factorial with three organic amendment placements and three nematode inoculum densities, replicated five times. The organic amendment treatment consisted of compost applied to the soil surface, mixed into soil, or no organic amendment. The organic amendment consisted of 190 cm^3 per pot of yard-waste compost, mainly woodchips, which was previously heated in an oven at 75 ^{o}C for 12 hours. The dry organic amendment weight was 45 g per pot. Subsamples of the compost were ground and screened for nutrient analysis. Nitrogen concentration was determined by using the aluminum block digestion method (Gallaher et al., 1975), P was determined by colorimetry, K by flame emission, and Ca, Mg, Cu, Fe, Mn, and Zn by atomic absorption spectrophotometry. The composition of the organic amendment was as follows: 0.39% N; 1,047 ppm P; 1,497 ppm K; 22,475 ppm Ca; 1,322 ppm Mg; 180 ppm Mn; 69 ppm Zn; 25 ppm Cu; and 770 ppm Fe. After 50 days, five soil samples from each of the amendment treatments were analyzed for pH, organic matter (Walkley and Black, 1934; Walkley, 1947), and soil extractable nutrients (Mehlich, 1953). Nitrogen was determined by using the aluminum block digestion method (Gallaher et al., 1975), analysis of P by colorimetry, K and Na by flame emission spectrophotometry, and Ca, Mg, Fe, Cu, Mn, and Zn by atomic absorption spectrophotometry.

The nematode inoculum consisted of second-stage juveniles (J2) of *Meloidogyne incognita* race 1. Eggs were extracted from roots of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) plants using 1.05% NaOCl (Hussey and Barker, 1973), and then incubated on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days. The

nematode densities used were 0, 250, and 1,000 J2/pot, applied one day after transplanting into three holes in the soil, about 2 cm deep.

The plant height was recorded 30 days after inoculation. At 50 days after inoculation, plants were harvested and the shoot and taproot lengths as well as the fresh and dry weights were recorded. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0= 0 galls or egg masses per root system, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100, and 5= more than 100 galls or egg masses per root system. Second-stage juveniles hatching from egg masses in the okra roots system were quantified by extraction in 1.05% NaOCl (Hussey and Barker, 1973), and then incubating eggs on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days and counting hatched J2.

Data were statistically analyzed as a completely randomized 3 x 3 factorial to determine the main effects and the interaction between organic amendment placement and nematode levels. When a main effect was significant ($P \leq 0.05$) with no interactions, a separate analysis of variance was carried out, followed by separation of means by Duncan's multiple-range test (Freed et al., 1987).

Results and Discussion

There were no significant interactions between organic amendment placement and inoculum density for top height and weight, root length and weight, and gall rating. However, there was a significant interaction ($P \leq 0.05$) between organic amendment placement and inoculum density for the egg mass rating (Table 2.1). Fewer egg masses

were observed in plants receiving either amendment treatment than in unamended plants at an inoculum level of 1,000 J2/pot, but not at 250 J2/pot (Table 2.2). Gall ratings did not show this trend. There was not enough evidence to determine if organic amendment placement had a significant effect on nematode population levels in soil (Table 2.3). Overall, organic amendment effects on nematode levels were inconsistent, with some trend toward lower numbers with organic amendment treatment.

Table 2.1. Effect of organic amendment placement and inoculum densities of *Meloidogyne incognita* race 1. F-values from the analysis of variance. April 1994.

Treatment effect	Plant height	Taproot length	Root weight	Top dry weight	Egg mass rating	Gall rating
Amendment placement (A)	34.97**	2.25 ns	88.29**	17.05**	6.77**	1.69 ns
Inoculum density (B)	3.38*	0.03 ns	0.81 ns	3.42*	9.38**	10.71**
Interaction (AxB)	0.38 ns	1.80 ns	0.32 ns	1.47 ns	3.87*	0.17 ns

*, ** Significant effects at P≤0.05 and P≤0.01, respectively; ns = not significant. Data are means of four replications.

Inoculum density had a significant effect (P≤0.05) on plant height as well as on top dry weight, and egg mass and gall rating. The same effect was observed for organic amendment placement. There were no significant differences among treatments relative to root length (Table 2.1).

Several plant parameters were affected by the experimental treatments (Table 2.1). Top height and weight were affected by the organic amendment placement as well as by inoculum density (Table 2.4). Top growth generally decreased as the nematode inoculum level increased. In general, organic amendment placed on the soil surface

Table 2.2 Effect of organic amendment placement and inoculum densities of *Meloidogyne incognita* race 1 on egg mass and gall ratings 50 days after inoculation. April 1994.

Organic amendment placement	250 J2/pot[§]		1,000 J2/pot	
	Egg masses[†]	Galls[†]	Egg masses	Galls
None	2.0 a[‡]	4.0 a	3.2 a	4.6 a
Surface	1.0 a	3.0 a	2.2 b	4.4 a
Incorporated	2.2 a	3.8 a	2.0 b	4.6 a

† Egg masses and galls rated on 0 to 5 scale, where 0= no galls; 1= 1 to 2 galls; 2= 3 to 10 galls; 3= 11 to 30 galls; 4= 31 to 100 galls; and 5= > 100 galls per root system.
‡ Data are means of five replications. Means followed by the same letter in a column are not significantly different (P≤0.05), according to Duncan's multiple-range test.
§ Number of juveniles hatched from the egg masses and used as inoculum.

Table 2.3. Effect of organic amendment placement and inoculum density of *Meloidogyne incognita* race 1 on numbers of juveniles (J2) hatched from the egg masses 50 days after inoculation. April 1994.

Organic amendment placement	250 J2/pot	1,000 J2/pot
	---------- J2 per g dry root ----------	
None	423[†]	904
Surface	482	459
Incorporated	1,009	1,184

† Statistical analysis was not performed because data are average of combined totals of four replications

provided the highest values of plant height and top weight. The lowest values occurred when the amendment was mixed with the soil. Root fresh weight was affected by organic amendment placement but not by inoculum density. The lowest root weights were observed when the organic amendment was mixed with the soil (Table 2.5). Root length was not affected by organic amendment placement or inoculum density (Table 2.5).

There was evidence that this organic amendment did not consistently benefit plant growth, in contrast to the results of Mankau (1968), Muller and Gooch (1982), and

Table 2.4. Effect of organic amendment placement and inoculum density of *Meloidogyne incognita* race 1 on plant height and dry shoot weight of okra. April 1994.

Organic	0 J2/pot‡			250 J2/pot			1,000 J2/pot		
amendment	Plant height (cm)		Weight	Plant height (cm)		Weight	Plant height (cm)		Weight
placement	30 days	50 days	(g)	30 days	50 days	(g)	30 days	50 days	(g)
None	19.20 a†	21.00 a	0.85 b	19.10 a	21.10 b	0.83 b	17.70 a	19.80 a	0.68 a
Surface	20.10 a	24.10 a	1.34 a	18.60 a	23.2 a	1.41 a	17.60 a	21.00 a	0.87 a
Incorporated	15.50 b	16.40 b	0.37 c	15.90 b	17.10 c	0.40 c	14.20 b	15.30 b	0.21 b

† Data are means of five replications. Means in columns followed by the same letter are not significantly different (P≤0.05), according to Duncan's multiple-range test.

‡ Number of juveniles hatched from the egg masses and used as inoculum.

Table 2.5. Effect of organic amendment on taproot length and root dry weight of okra 50 days after inoculation. April 1994.

Organic amendment placement	Taproot length (cm)	Weight (g)
None	25.50 a†	0.48 b
Surface	21.30 a	0.82 a
Incorporated	19.00 a	0.28 c

† Data are means of 15 replications. Means followed by the same letter in columns are not significantly different (P≤0.05), according to Duncan's multiple-range test.
‡ Number of juveniles hatched from the egg masses and used as inoculum.

Trivedi and Barker (1986). However, it was evident that mixing the amendment with soil produces a negative growth response compared to amendment applied to the soil surface. According to Reuszer (1957), an amendment of this nature, high in lignin and cellulose, may require extra N and P when it is mixed with the soil. Therefore, when incorporated, the amendment will compete for nutrients much more than when the amendment is applied on the soil surface. Research has shown that microorganisms may use great amounts of N to decompose organic amendments and that incorporation of organic matter high in C may create N-deficient soil for the plants (Gallaher and McSorley, 1994). The soil extractable nutrient analyses (Table 2.6) revealed high levels of organic matter and N when yard-waste compost was added to the soil, either on the soil surface or mixed. Nonetheless, in general, higher values of P, Mg, Mn, Cu, and Na were found when the amendment was placed on the soil surface (Table 2.6), while other nutrientes (Ca, Zn, Fe) were unaffected. Organic residues with low N require much more time to be decomposed, producing less humus than organic residues with higher N level. Therefore, it has been suggested that supplemental N application may increase microbial

Table 2.6. Organic matter, pH and extractable nutrients in soil, after 50 days. April 1994.

Treatments[‡]	OM	N	pH	P	K	Mg	Ca	Mn	Zn	Cu	Fe	Na
	%			ppm								
None	0.60 b[†]	0.0054 b	7.92 a	105 b	107 b	66 c	5328 a	3.3 c	18.7 a	0.32 a	4.24 a	38.6 ab
Surface	1.23 a	0.0158 ab	7.76 b	118 a	49 c	94 a	5408 a	5.8 a	20.9 a	0.21 ab	4.24 a	41.0 a
Incorporated	1.31 a	0.0176 a	7.72 b	95 b	254 a	78 b	5488 a	4.8 b	28.2 a	0.18 b	2.64 a	33.6 b

[†] Data are means of four replications. Means followed by the same letter in columns are not significantly different (P≤0.05), according to Duncan' s test.

[‡] Organic amendment placement.

activity and hasten the decomposition of amendments with high C/N ratios (Holtz and Vandecaveye, 1938).

Rodríguez-Kábana (1986), working with organic amendments with different C/N ratios, reported that when more N was available, nematode control was enhanced. These results may have been due to the fact that some organic amendments require much time to be decomposed and release N or other products. According to McSorley and Gallaher (1995b), even after 4-4.5 months, yard-waste compost treatment had no impact on a nematode population, and a longer time period may be required for this organic amendment to have an effect.

The manner in which organic amendments affect nematodes is complex and remains poorly understood. Stirling (1991) suggested that amendments which are high in N and have a balanced C:N ratio act by producing nematoxic levels of ammonia. This is not the case with yard waste compost (mainly woodchips) (Gallaher and McSorley, 1994; 1995a, b). Stirling (1991) suggests that nematicidal activity of wastes with high C:N ratios can be improved by adding urea or other sources of N.

Under the conditions of this experiment, addition of the organic amendment did not consistently affect development of *Meloidogyne incognita* race 1. However, a beneficial effect on plant growth was observed when the organic amendment was placed on the soil surface, rather than mixed with soil, even at a high nematode level.

CHAPTER 3
EFFECT OF PLANTING DATE AND INOCULUM DENSITY OF *MELOIDOGYNE INCOGNITA* ON OKRA

Introduction

Okra (*Hibiscus esculentus* L.) is a small-farm vegetable crop commonly grown on many Florida soils. However, several phytosanitary problems can reduce okra quality and yields (William et al., 1982). Among them, the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood is a pest of okra (Christie, 1959).

Development and reproduction of *M. incognita* depend upon different physiological and environmental factors such as crop susceptibility, nutritional condition of the host plant , and soil temperature (Davide and Triantaphyllou, 1967; Roberts et al., 1981). For these reasons, cultural control practices may result in inconsistent reductions of populations. Yield of susceptible plants depends on the nematode species and density (Seinhorst, 1965, 1966, 1967). The rate of growth of a nematode population has been measured in several crops at different densities or plant nutritional stages (Seinhorst, 1965, 1966, 1967; Nusbaus and Ferris, 1973) because this information can be useful for proper crop management.

Time of planting has been used as an important strategy to suppress nematode infestation (Roberts et al., 1981). However, for some crops it cannot be used (Todd, 1993) since the nematode damage and reproduction rate depend on nematode species and crop susceptibility, or crop tolerance to environmental stress during nematode

reproduction. For field management of nematode populations, knowledge of the relationship between planting date and damage may result in more choices for growing various crops at lower risk (Roberts et al., 1981; Brown, 1987; Rodríguez-Kábana, 1986; Trivedi and Barker, 1986). However, there are not enough data on the dynamics of *M. incognita* on okra to determine if changing the planting date will be effective in reducing nematode populations.

The objectives of this study were to examine the effect of three different inoculum concentrations of *M. incognita* race 1 and five different planting dates of okra on nematode population densities in soil and growth of okra under greenhouse conditions.

Materials and Methods

The experiment was carried out in the spring of 1994 in a greenhouse on the University of Florida campus in Gainesville, Florida. Plastic pots 10 cm in diameter and 12 cm high were filled with 700 cm^3 of steam-sterilized field soil mixed with sand, in a ratio of 1:1 by volume. The composition of the mixture was 92% sand, 4% clay, and 4% silt. The dry weight of the soil mixture was 740 g per pot.

The experimental design was a 3 x 5 factorial with three nematode inoculum densities and five planting dates, replicated five times. The nematode inoculum consisted of second-stage juveniles (J2) of *Meloidogyne incognita* race 1. Eggs were extracted from roots of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) plants using 1.05% NaOCl (Hussey and Barker, 1973), and then incubated on Baermann trays (Rodríguez-Kábana and Pope, 1981) at room temperature for seven days. The hatched J2 were

counted and used as inoculum. The nematode densities used were 0, 250, and 1,000 J2/pot.

Okra seedlings were maintained in a growth room, where seeds were individually sown in Speedling trays (27 cm x 52 cm, with capacity for 36 seedlings) at five different times: 17 January (three weeks); 24 January (two weeks); 31 January (one week); 6 February (germinated seed); and 7 February (seed). For all times except 6 February, germinated seedlings were obtained from seeds placed in moist, sandy soil one day earlier. Thus, the treatment time can be denoted as 21, 14, 7, 1, or 0 days, respectively. The Speedling trays were kept at 24 ± 1 °C and were watered daily.

On 6 February, three-, two-, and one-week-old seedlings of the okra cultivar 'Clemson Spineless' were individually transplanted into the pots, after applying 0.6 g of granular 6-6-6 (N-P-K) fertilizer. The pots were watered immediately after transplanting. On 7 February, seed or newly germinated seedlings (depending on the treatment) were individually sown 2 cm deep into mixed soil in similar pots. Each pot received the appropriate inoculum treatment, which was applied in three holes in the soil, about 2 cm deep, one day after transplanting. Plants were sprayed two times a week with dilute soap solution to reduce infestation of whiteflies (*Bemisia tabaci*).

The average minimum and maximum soil temperatures were 19.4 ± 3.2 °C and 28.9 ± 3.5 °C, respectively, in February, and 16.2 ± 5.5 °C and 28.5 ± 2.9 °C in March. The average minimum and maximum air temperatures in the greenhouse were 13.5 ± 2 °C and 38.0 ± 2.5 °C in February, and 15.5 ± 1.8 °C and 42.1 ± 1.8 °C in March.

Thirty days after inoculation, the plant height was recorded. Fifty days after inoculation, plants were harvested, and the shoot and taproot lengths as well as the fresh

and dry weights were recorded. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0= 0 galls or egg masses per root system, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100, and 5= more than 100 galls or egg masses per root system.

The number of second-stage juveniles hatching from egg masses in the okra root system was quantified by extraction of eggs in 1.05% NaOCl (Hussey and Barker, 1973), and then incubating eggs on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days and then counting the hatched J2. The centrifugal flotation technique (Jenkins, 1964) was utilized to quantify J2 from 100 cm^3 soil. However, data on J2 extracted from the egg masses and from the soil were not statistically analyzed since they were recorded based on the average of the four root systems or two soil samples, respectively.

Data were statistically analyzed as a completely randomized 3 x 5 factorial design to determine the main effects and the interaction between inoculum density and planting date. When a main effect was significant with no interactions, a separate regression analysis was carried out (SAS Institute, Cary, NC) to determine if the averages across the three inoculum levels followed a linear relationship with planting date. Least-squares means (LSMEANS) analysis was used to summarize the effects since the numbers of replications varied throughout the study. For the egg mass and gall rating variables, data obtained from seed at time 0 were not included in analyses because of missing data.

Results and Discussion

There were no significant interactions between inoculum density and planting date for top height at 30 and 50 days, top weight, taproot length and weight, egg mass rating, and gall rating (Table 3.1). A significant main effect of planting date was observed for most plant parameters and for egg mass rating ($P \leq 0.05$), but not for gall rating ($P \geq 0.05$) (Table 3.1). Inoculum density had a significant effect on gall rating and root weight ($P \leq 0.01$).

Plant height and top weight generally increased with planting date. Plants grown from seed or germinated seed were not uniform in their development and germination. Plant growth in those planting dates was poor regardless of the treatment (Table 3.2). Nonetheless, there was not enough evidence to conclude if the lack of germination was due to the variability of light and temperature in the greenhouse, where environmental conditions were less controlled than those in the growth room. Because of the high variability from these two planting dates, analysis of variance was repeated using only planting dates of 21, 14, and 7 days. Results with three planting dates (Table 3.3) were similar to those achieved when all five planting dates were used (Table 3.1). The poor stands generated by the 0 and 1 day treatments appeared not to affect the significance of most plant parameters, but the interaction between density and planting date was significant ($P \leq 0.05$) for root weight (Table 3.3).

Plant height at 30 or 50 days after inoculation showed a positive linear relationship ($P \leq 0.01$) with the increase of planting date (Figure 3.1), regardless of the

Table 3.1. Effect of inoculum density of *Meloidogyne incognita* race 1 and planting date on growth of okra and egg mass and gall ratings. F-values from the analysis of variance.

Treatment	Plant height		Top weight		Taproot	Root weight		Egg mass	Gall
effect	30 days	50 days	fresh	dry	length	Fresh	Dry	rating	rating
Inoculum density (A)	2.37 ns	1.82 ns	0.49	0.51 ns	0.86 ns	7.56**	7.48**	0.45 ns	19.02**
Planting date (B)[†]	60.01**	23.84**	7.32**	7.34**	6.71**	12.18**	11.97**	3.63*	1.03 ns
Interaction (AxB)	1.71 ns	0.99 ns	1.21 ns	1.23 ns	0.70 ns	1.68 ns	1.66 ns	0.48 ns	2.09 ns

*, ** Significant effects at P≤0.05 and P≤0.01, respectively; ns = not significant.

[†] Planting dates include 21, 14, 7, 1, and 0 days.

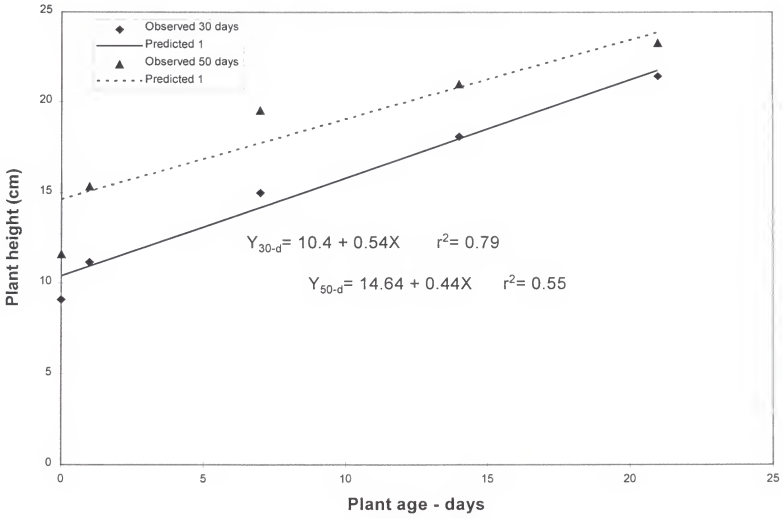

Figure 3.1. Relationship between plant height at 30 and 50 days after inoculation and plant age in okra. Each point along the regression line is the average of 0, 250, or 1,000 J2/pot for each planting time. Data are average of final stand based on numbers in Table 3.2. April 1994.

inoculum density (P≤0.01). Top fresh and dry weight and taproot length also increased with plant age regardless of the inoculum density (P≤0.01) (Data not shown).

Table 3.2. Effect of inoculum density of *Meloidogyne incognita* and planting time (days before inoculation) on final stand of okra. April 1994.

Planting time (days)	0 J2/pot†	250 J2/pot	1,000 J2/pot
	---------- Number of pots with okra ----------		
21	5	5	5
14	5	5	5
7	5	5	5
1	2	2	1
0	2	2	2

† Number of juveniles (J2) hatched from the egg masses and used as inoculum.

Nematode inoculum densities did not affect plant growth, except for root fresh and dry weight. The lowest root weight was observed when there was no inoculum, and the highest weight was observed at the highest inoculum density. Root weight also increased positively with planting date. For fresh root weight, the relationships were best described by linear regression equations (P≤0.01) for the three inoculum levels (Figure 3.2). Similar relationships were obtained with root dry weight (Data not shown). The fresh and dry root weights were greatest at the highest inoculum level for the three-week-old plants, perhaps due to the increased number and size of root galls. This trend agrees with data presented by Gaur et al. (1979), who found a positive relationship between fresh root weight and nematode level due to an increase in the root-knot index.

No juveniles hatched from egg masses and none were extracted from soil when plants were grown from germinated seed or seed (Table 3.4). One possible reason was the fact that nematode inoculum was available but there were no feeding sites because

Table 3.3. Effect of inoculum density of *Meloidogyne incognita* race 1 and planting date on growth of okra and egg mass and gall ratings. F-values from the analysis of variance.

Treatment	Plant height		Top weight		Taproot	Root weight		Egg mass	Gall
effect	30 days	50 days	fresh	dry	length	Fresh	Dry	rating	rating
Inoculum density (A)	0.35 ns	1.07 ns	0.66	0.67 ns	1.54 ns	10.90**	10.82**	0.08 ns	10.53**
Planting date (B)†	46.42**	7.83**	3.61**	3.64*	1.99 ns	12.21**	11.77**	2.96 ns	1.37 ns
Interaction (AxB)	0.91 ns	0.70 ns	2.16 ns	2.19 ns	1.09 ns	3.41*	3.33*	0.56 ns	1.37 ns

*, ** Significant effects at P≤0.05 and P≤0.01, respectively; ns = not significant.

† Planting dates include 21, 14, and 7 days.

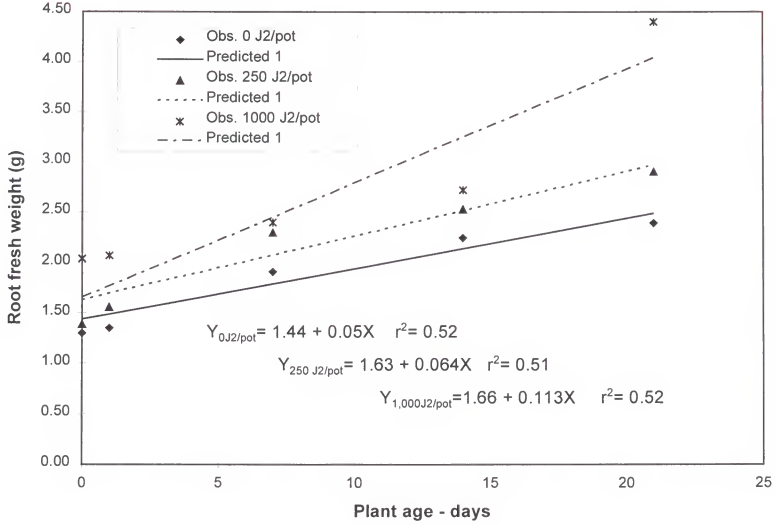

Figure 3.2. Relationship between root fresh weight and plant age in okra. Each point along the linear regression is the average of the plant numbers remaining (see Table 3.2). April 1994.

Table 3.4. Effect of inoculum density of *Meloidogyne incognita* race 1 and planting time (days before inoculation) on numbers of juveniles (J2) hatched from egg masses, and on numbers of juveniles (J2) in 100 cm^3 of soil, on okra 50 days after inoculation. April 1994.

Planting	J2 per g dry root†		J2 per 100 cm^3 soil†	
time (days)	250 J2/pot‡	1,000 J2/pot	250 J2/pot	1,000 J2/pot
21	771	1,982	3	5.5
14	2,463	2,105	6.5	2.5
7	419	1,140	0	2
1	0	0	0	0
0	0	0	0	0

† Statistical analysis was not performed because data are average of combined totals of four replications.

‡ Number of J2 used as inoculum.

the root system was not well developed. However, depending on the inoculum density, inoculation of three-week-old seedlings with 250 J2/pot resulted in almost twice as many juveniles hatching from the egg masses as with one-week-old seedlings, while two-week-old seedlings yielded about six times more J2 than one-week-old seedlings. At the highest inoculum density, the number of hatched juveniles was more than double the number from the lower inoculum density, but not for two-week-old seedlings (Table 3.4). According to Roberts et al. (1981), nematode development is dependent on soil temperature, which affects nematode movement and infection. The majority of nematodes reach the adult stage 19 to 25 days after inoculation at 20 ^{0}C or 38 to 50 days at 15 ^{0}C (Davide and Triantaphyllou, 1967). Egg production at 15 ^{0}C will occur after 60 days. During the 50 days of the greenhouse study, much fluctuation in soil temperature was observed. Possibly, the variability of minimum soil temperatures in pots of $\pm$ 3.2 ^{0}C in February and $\pm$ 5.5 ^{0}C in March may explain the variability in numbers of juveniles hatched from egg masses and extracted from the 100 cm^3 soil samples.

The number of juveniles extracted from 100 cm^3 of soil was very low (Table 3.4). It is possible that the second generation was not completely developed, because the temperature was cool during the last 20 days before harvesting the experiment. In addition, plants could not remain in pots much longer because there was insufficient space for the root system to further develop and support plant growth.

Davide and Triantaphyllou (1967) stress that the age of the host plant does not substantially influence either the rate of nematode development or the sex ratio because the root-knot nematodes establish themselves at the growing apical meristems of the roots, which may not be affected by the age of the host. However, the date of sowing has a profound influence on plant growth and size, and therefore presumably on the number of infection sites for nematodes. Of course, other factors which influence nematode infection, including host susceptibility and environmental conditions (particularly temperature and moisture) Gaur et al., (1979), McSorley and Gallaher (1992), would need to remain relatively constant.

The fluctuation of soil temperatures might influence the developmental cycle of the nematode as well as plant growth. Nonetheless, based on the greenhouse conditions in this test, two-week-old okra seedlings were considered best for building up nematode populations and studying nematode population dynamics.

CHAPTER 4
EFFECT OF MULCH, IRRIGATION FREQUENCY, AND INOCULUM LEVEL OF *MELOIDOGYNE INCOGNITA* ON 'CLEMSON SPINELESS' OKRA

Introduction

Organic matter may promote plant growth by improving soil fertility, preventing erosion, maintaining soil moisture, or facilitating conditions for development of nematode-antagonistic microorganisms (Watson, 1943; Mankau, 1968; Muller and Gooch, 1982; Trivedi and Barker, 1986). The maintenance of favorable soil moisture by mulch may minimize plant stress. Watson (1945) observed that plants suffering from drought were always more seriously affected by root-knot nematodes than those which received plenty of moisture, and that mulched plants had more healthy roots than unmulched plants. However, the organisms responsible for the decay of organic matter are voracious feeders on N, and when supplied with a C source, they may take up all the available N in the soil and cause growing plants to suffer temporarily from a lack of this element (Watson, 1943). However, it is important to know the quality of the mulch and the C/N ratio before making inferences, since fully decayed organic mulches add N and organic matter to the soil, improving its nutrient content and water-holding capacity (Watson, 1943). Nusbaum and Ferris (1973) emphasized that stresses on crop growth, such as low soil moisture or fertility, might unbalance the system, causing fluctuations of nematode populations. However, this general information cannot easily be applied to

different crops or nematodes, because there are so many specific interactions for each crop, nematode species, and environmental factors such as moisture and fertility.

The objective of this preliminary study was to examine the effect of two irrigation periods under three different types of mulch, plus a control treatment (without mulch) on two concentrations of *Meloidogyne incognita* (Kofoid and White) Chitwood race 1 on okra (*Hibiscus esculentus* L.), in order to determine the effect of mulch type, inoculum level, and irrigation period on plant growth.

Materials and Methods

Seeds of the okra cultivar 'Clemson Spineless' were individually sown in Speeding trays (27 cm x 52 cm, with capacity for 36 seedlings) in a growth room. These trays were filled with steam-sterilized sandy soil (92% sand, 4% silt, 4% clay), kept at 24 ± 1 °C, and were watered daily. After two weeks, on 26 June 1994, these seedlings were individually transplanted into pots (10 cm in diameter and 9 cm high) containing the steam-sterilized field soil and sand at a ratio of 1:1 by volume. Pots were watered to field capacity immediately after transplanting. Plants were fertilized by applying 0.6 g of granular 6-6-6 (N-P-K) fertilizer to each pot. Each pot held 700 cm^3 of soil, which weighed 740 g.

On 28 June, these plants were inoculated with nematodes. The inoculum consisted of fresh second-stage juveniles (J2) of *Meloidogyne incognita* race 1. Egg masses were extracted from roots of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) plants with 1.05% NaOCl (Hussey and Barker, 1973) and then incubated on Baermann

trays for seven days following the technique of Rodríguez-Kábana and Pope (1981). The inoculum densities used were 0 and 1,000 second-stage juveniles per pot. After inoculation, mulch was placed individually on each pot according to the required treatment. The mulch treatments consisted of 190 cm^3 of perennial peanut (*Arachis glabrata*) hay weighing 19.05 g; woodchips from an aged yard-waste compost (McSorley and Gallaher, 1995b) weighing 24.10 g; pieces of Styrofoam plastic (about 1 cm diameter) weighing 0.95 g; and a control treatment (without mulch). The peanut hay and woodchips, previously dried to constant weight, were kept overnight in an oven at about 75 ^{0}C before application, to prevent or delay fungal or bacterial growth. The C/N ratio was 10.66 for peanut hay, and 14.78 for woodchips. The irrigation treatments consisted of 25 ml of water every two days or 25 ml of water every four days. The experimental design was a 4 x 2 x 2 factorial, with four mulch types, two inoculum concentrations, and two irrigation times, replicated eight times. For each treatment combination, three replications were used for determination of soil moisture content. With a 7-mm-diameter cork borer, soil samples were taken before and after irrigation, at two and four days. These samples were immediately weighed using a precise scale, dried in oven until constant dry weight was reached, and then weighed again. The soil moisture levels were estimated four times at weekly intervals. At each sampling, the soil sample from a pot was obtained by placing the cork borer at a different position in relation to the pot label, so that the same pot would not be sampled twice at the same place.

Data on plant height and stem diameter were taken at 23 and 36 days after inoculation. Forty-two days after inoculation, plants were harvested and the following

data were recorded for each replication: shoot and taproot length, and fresh and dry weight of shoot and root. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0= 0 galls or egg masses per root system, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100, and 5= more than 100 galls or egg masses per root system. The final population of second-stage juveniles in soil and in egg masses were also recorded but for only two and four replications, respectively. The centrifugal flotation technique (Jenkins, 1964) was used to quantify juveniles (J2) from 100 cm^3 of soil, and the J2 from eggs in the root system were quantified with the Baermann incubation technique (Rodríguez-Kábana and Pope, 1981).

Data were statistically analyzed (SAS Institute, Cary, NC) as a completely randomized 4 x 2 x 2 factorial design with five replications for each treatment, to determine the main effects or interactions of mulch, irrigation frequency, or nematode level. For comparisons among means, a special contrast with least-squares means (LSMEANS) was used because in some cases there were cells with no data (dead plants) in the experimental design or because the numbers of replications varied throughout the study.

Results and Discussion

There was a significant interaction ($P \leq 0.05$) between inoculum level and irrigation frequency for plant height at 23, 36, and 42 days after inoculation (Table 4.1). In general, plants watered every four days were consistently smaller than those watered

Table 4.1. Effect of inoculum level, irrigation frequency, and mulch type on 'Clemson spineless' okra in greenhouse experiments. August 1994. Data shown are F-values from the analysis of variance.

Treatment	Plant height			Stem diameter		Top weight		Taproot	Root weight	
effect	23 days	36 days	42 days	23 days	36 days	Fresh	Dry	length	Fresh	Dry
Inoculum level (A)	1.11 ns	1.00 ns	0.21 ns	4.68*	2.94 ns	3.46 ns	4.17*	5.46*	20.14**	78.72**
Irrigation frequency (B)	52.65**	56.77**	82.51**	67.02**	83.39**	280.24**	191.74**	0.37 ns	85.67**	161.46**
Mulch type (C)	1.09 ns	0.96 ns	1.80 ns	14.58**	16.25**	19.62**	11.30**	0.30 ns	9.70**	8.36 ns
Interaction (AB)	6.76*	5.63*	6.66*	0.06 ns	0.13 ns	0.80 ns	1.39 ns	2.16 ns	3.56 ns	9.66**
Interaction (AC)	0.21 ns	0.22 ns	0.07 ns	1.79 ns	2.58 ns	1.89 ns	0.48 ns	0.63 ns	3.18*	47.58**
Interaction (BC)	2.27 ns	2.03 ns	1.99 ns	2.11 ns	1.78 ns	1.28 ns	1.17 ns	4.04*	2.22 ns	29.84**
Interaction (ABC)	0.14 ns	1.51 ns	1.11 ns	0.06 ns	0.19 ns	0.26 ns	0.01 ns	0.35 ns	0.13 ns	0.04 ns

*, ** Significant effects at P≤ 0.05 and P≤ 0.01, respectively; ns= not significant.

every two days (Table 4.2). Although irrigation frequency consistently affected plant height, this effect was more severe in the presence of the nematode (Table 4.2).

There was a significant main effect ($P \leq 0.01$) of irrigation frequency on stem diameter, and on top fresh and dry weight. In general, irrigation every two days resulted in higher values for all of these plant parameters (Table 4.3).

There was a significant main effect of mulch type on stem diameter, and on top fresh and dry weight. In general, those plant parameters showed higher values under mulch. Stem diameter was affected by the inoculum level ($P \leq 0.05$), irrigation frequency ($P \leq 0.01$), or mulch type ($P \leq 0.01$), but no interaction was observed ($P \geq 0.05$). Plants that were infected by the nematodes had a smaller stem diameter, as well as plants that were irrigated every four days (Table 4.3). Plants that received mulch had a larger stem diameter than plants that did not receive mulch (Figure 4.1). Top weights showed similar trends (Figure 4.2).

Table 4.2. Effect of irrigation frequency and inoculum level of *Meloidogyne incognita* juveniles (J2) on plant height of okra. August, 1994.

Irrigation frequency	Plant height (cm)	
	0 J2/pot	1,000 J2/pot
	23 days after inoculation	
2 days	35.78 a[†]	37.74 a
4 days	28.95 b	24.43 b
	36 days after inoculation	
2 days	51.01 a	53.59 a
4 days	39.95 b	33.81 b
	42 days after inoculation	
2 days	54.49 a	58.27 a
4 days	41.16 b	35.81 b

[†] Within each harvest time, means in columns (a, b) followed by the same letter do not differ at $P \leq 0.05$, according to LSMEANS analysis.

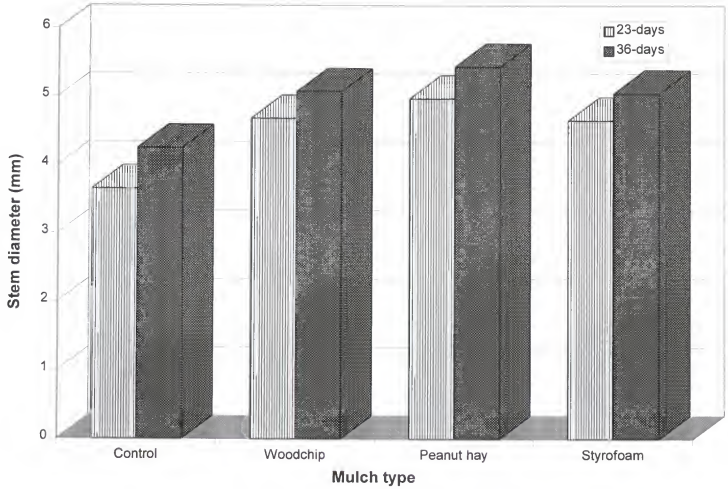

Figure 4.1. Effect of mulch type on stem diameter of okra.

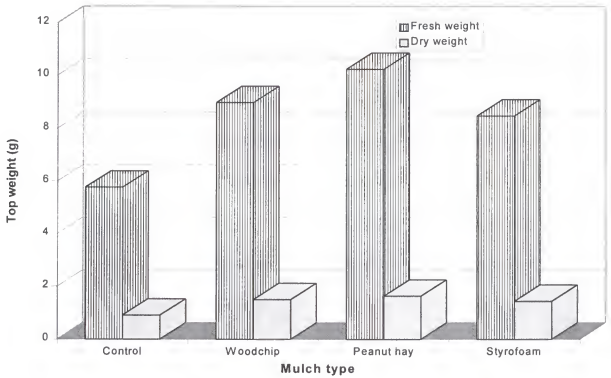

Figure 4.2. Effect of mulch type on fresh and dry top weight of okra.

Taproot dry weight was also affected by the interaction between inoculum level and irrigation (P≤0.01). The root dry weight was lower when plants were watered every four days (Figure 4.3). However, at 1,000 J2/pot, the root weight was higher than at 0 J2/pot. This might be due to the fact that plants infected by the root-knot nematode had

Table 4.3. Effect of irrigation frequency and inoculum level of *Meloidogyne incognita* juveniles (J2) on stem diameter and top dry weight of okra. August, 1994.

Irrigation	Stem diameter (mm)		Top dry weight (g)	
frequency	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot
2 days	5.09[†] a	4.84 a	1.91 a	1.86 a
4 days	4.11 b	3.91 b	0.96 b	0.75 b

[†] Within each harvest time, means in columns (a, b) followed by the same letter do not differ at P≤0.05, according to LSMEANS analysis.

swollen roots and galls which contributed to the increase in root weight.

There was a significant interaction between inoculum level and mulch type for fresh (P≤0.05) and dry weight of roots (P≤0.01). The benefit of using mulch is evident because for development and growth of the root system at 0 J2/pot, the best root development was observed under mulch treatments. In the presence of the nematode, the highest root weight was observed in the no-mulch treatment due to the presence of the swollen roots (Figure 4.4). When mulch was used, there was little difference in root dry weight between the 0 and 1,000 inoculum levels (Figure 4.4). Data on fresh root weights showed similar trends (data not shown).

There were significant interactions between irrigation frequency and mulch type for root dry weight (P≤0.01) and taproot length (P≤0.05). The beneficial effect of using mulch when plants were watered every four days is evident from comparison of mulched

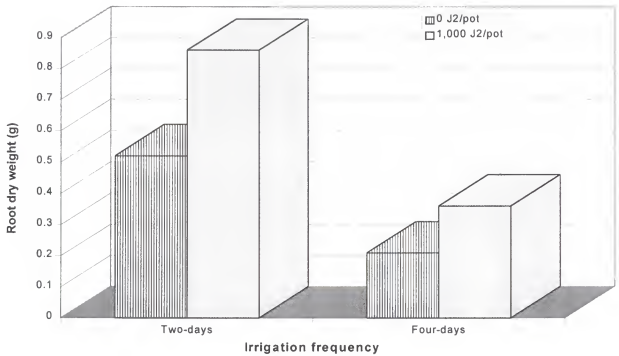

Figure 4.3. Effect of irrigation frequency and inoculum level (*Meloidogyne incognita*) on root dry weight of okra.

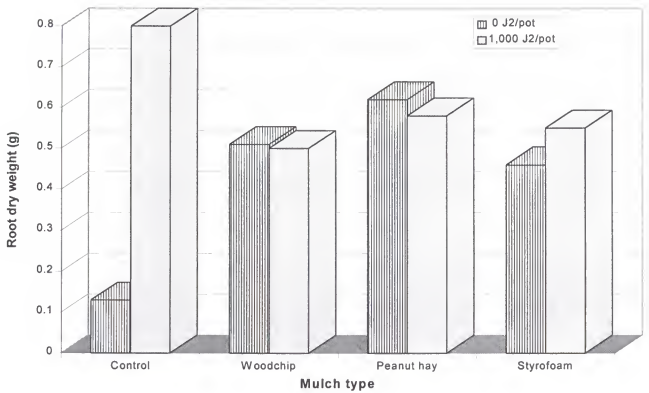

Figure 4.4. Effect of mulch type and inoculum level (*Meloidogyne incognita*) on root dry weight of okra.

treatments with the control (Figure 4.5). Nonetheless, root weight was not different among the different types of mulch (Figures 4.4, 4.5).

Egg mass ratings were affected by the irrigation frequency ($P \leq 0.05$) and mulch type ($P \leq 0.05$), but no interaction was observed ($P \geq 0.05$) (Table 4.4), and egg mass ratings were low (<0.8) in all cases (data not shown). Apparently, gall index was not affected by treatments. However, when juveniles were extracted from the root systems, the highest numbers were observed in the no mulch and woodchip treatments with two-day irrigation (Table 4.5). Nonetheless, it seems that there was no difference when the irrigation time was set at four days (Table 4.5). When juveniles were extracted from soil, the samples from no-mulch and woodchip treatments were so dirty that the nematodes could not be counted. The styrofoam treatment had higher juvenile numbers than the peanut hay treatment (data not shown).

For the soil moisture measurements, statistical analysis was not performed since many plants died after soil samples were taken, and soil moisture data over time were somewhat erratic (data not shown). However, it was possible to observe that plants were severely affected by dryness, and root systems were drastically damaged. For the first week, soil moisture was relatively stable in pots that were watered two days as well as those watered every four days. This may be due to the small demand of plants for water because plant size was so small. However, as the plants developed, plants that received water every two days developed faster and demanded more water than plants that were watered every four days. Thus, the four-day treatment had lower plant development due to the plant drought stress. Consequently, both plant growth and soil moisture were

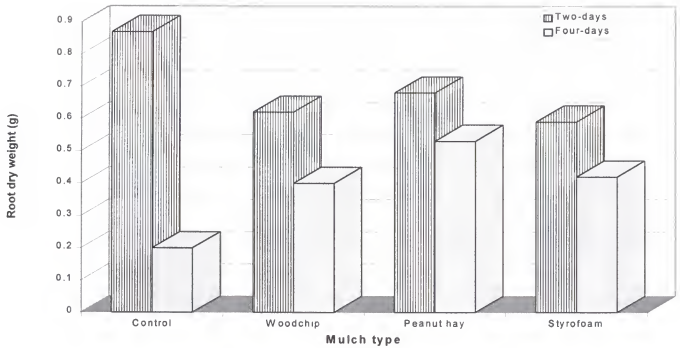

Figure 4.5. Effect of mulch type and irrigation frequency on root dry weight of okra.

Table 4.4. Effect of irrigation frequency and mulch type on egg masses and gall formation of *Meloidogyne incognita* race 1 on roots of okra. Data shown are F-values from the analysis of variance. August 1994.

Treatment effect	Egg masses	Galls
Irrigation frequency (A)	5.08 *	2.36 ns
Mulch type (B)	4.28 *	0.34 ns
Interaction (AB)	1.83 ns	1.34 ns

*, ** Significant effects at P≤0.05 and P≤0.01, respectively; Ns = not significant.

Table 4.5. Effect of irrigation frequency and mulch type on numbers of juveniles (J2) hatched from the egg masses 42 days after inoculation. August, 1994.

Mulch type	Irrigation frequency	
	2 days	4 days
	---------- J2/ g root ----------	
None	137[†]	8
Woodchip	216	11
Peanut hay	30	11
Styrofoam	92	21

[†] Statistical analysis was not performed because data are average of the remaining plants of the 1,000 J2/pot treatment. For some treatments, the root system was damaged or not in good condition for extracting nematodes.

lower. This may have resulted in reduced root systems, so that even though water was supplied, the plants were not able to make use of it. This may explain why some pots in the four-day treatment had higher soil moisture at some point during the experiment than those with two-day irrigation frequency. Regardless of measured trends in soil moisture in pots over time, the effect of the reduced irrigation frequency on plant growth was great.

In general, there was not much difference in plant growth measures among mulch types within irrigation or inoculum treatments, but there was a great difference between the mulch types and the unmulched control. There is evidence that mulch improves plant growth, probably due to water conservation, since other parameters were kept constant under greenhouse conditions. In addition, it was evident that irrigation frequency affected plant performance and that nematodes negatively affected plant growth.

CHAPTER 5

EFFECT OF FRESH AND DRY ORGANIC AMENDMENTS ON *MELOIDOGYNE ARENARIA* IN GREENHOUSE EXPERIMENTS

Introduction

Organic amendments have been used by farmers and researchers to manage plant-parasitic nematodes since early in this century, but they have not been fully accepted or understood as a biological control method because of the many interactions with environmental factors (Linford et al., 1938; Watson, 1945; Mankau, 1968). The mechanism of action has been attributed to the improvement of soil structure and aggregation, resulting in increasing aeration and water-holding capacity, to improvement in plant nutrition, or to the enhancement of the growth of organisms able to compete with or destroy nematodes (Stirling, 1991). These attributes might be considered in isolated cases since the efficacy of organic amendments has varied depending upon the nature of the organic matter, soil type, crop, and nematode species.

A number of organic amendments have been used to manage nematodes, because they are associated with reduced infection or survival of the nematode species, or because they may increase microbial and animal species antagonistic to nematodes (Linford et al., 1938; Watson, 1945; Mankau and Minteer, 1962; Mankau, 1968; Sitaramaiah and Singh, 1978; Rodríguez-Kábana et al., 1989). These effects may have resulted from the release

of phenolic compounds, NH_3 or nitrite, Ca^+ ions, from changes in soil pH or soil moisture, or from the type, amount, and C/N ratio of the organic amendment (Holtz and Vandecaveye, 1938; Watson, 1945; Mankau and Minteer, 1962; Singh and Sitaramaiah, 1967, Mankau, 1968; Walker, 1971; Rodríguez-Kábana, 1986; Brown, 1987). Nonetheless, the dynamics of the nematode population under organic amendments may not be related to better yields, since the organic amendment itself may promote healthy plants, as well as improvement in soil moisture. Thus, some crops may better tolerate the presence of nematodes, without showing decreased yields (McSorley and Gallaher, 1995).

Castor (*Ricinus communis* L.), collard (*Brassica oleracea* L.), sesame (*Sesame indicum* L.), sorghum-sudangrass 'SX-17' (*Sorghum bicolor* [L.] Moench x *Sorghum sudanense* [Stapf] Hitchc.), velvetbean (*Mucuna deeringiana* [Bort.] Merr.), and zinnia (*Zinnia elegans* L.) were chosen for greenhouse studies, because they have been previously used as organic amendments to suppress nematodes when used in crop rotation, incorporated into soil, or applied as mulch (Watson, 1922, 1936, 1945; Watson and Goff, 1937; Lear, 1959; Mankau and Minteer, 1962; Mankau, 1968; Mian and Rodríguez-Kábana, 1982; Huang, 1984; Rodríguez-Kábana, 1986; Vicente and Acosta, 1987; Rodríguez-Kábana et al., 1988, 1989, 1992; Rich et al., 1989; McSorley and Gallaher, 1993; McSorley et al., 1994). The objective of these greenhouse experiments was to compare the effect of fresh and dry material of these six organic amendments on okra (*Hibiscus esculentus* L.) inoculated with *Meloidogyne arenaria*, (Neal) Chitwood race 1.

Materials and Methods

Two separate experiments were carried out during the spring and summer of 1995, in a greenhouse on the University of Florida campus in Gainesville, Florida. The following plants were grown to supply fresh or dry organic amendments for both experiments: castor, 'Georgia Southern' collard, 'Sesaco-16' sesame, 'SX-17' sorghum-sudangrass, velvetbean, and 'Scarlet' zinnia. Plants were grown in clay pots of 10 cm diameter and fertilized by applying 2.5 g of a granular 6-6-6 (N-P_2O_5-K_2O) fertilizer to each pot at planting. Aboveground parts of all plants were harvested before they reached the reproductive stage. Leaves and stems of each one were chopped separately, then well mixed, and used as fresh or dry amendment, as specified for each experiment. A sample of each type of plant was taken for mineral analysis and C/N ratio as previously described according to the methodology in chapter 2 (Gallaher et al., 1975; Walkley and Black, 1934; Walkley, 1947; Mehlich, 1953). Results obtained are presented in Table 5.1.

For the inoculum treatment, a soil infested with *Meloidogyne arenaria* race 1 was used. This soil had been infested by adding the nematode to steam-sterilized field soil, followed by growth of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.). For the no-inoculum treatment, a soil where 'Rutgers' tomato was grown without *M. arenaria* race 1 was used. The final soil mixture was prepared by mixing 1/4 infested soil and 3/4 of steam-sterilized field soil mixed with sand (in a ratio of 1:1 by volume). The soil mixture for the no-inoculum treatment consisted of 1/4 of non-infested soil, and 3/4 of steam-sterilized field soil mixed with sand (1:1). Soil samples were taken from both batches to determine the initial nematode population levels. The initial inoculum level of second-

Table 5.1. Plant mineral analysis and C/N ratio for each organic amendment. Data are means of five replications, from the aerial plant parts harvested before the reproductive stage.

Organic amendment	C/N ratio	Macronutrients					Micronutrients			
		N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
		--- % ---	----------	----------	----------	----------	ppm	----------	----------	----------
Castor	7.91	2.26	3,400	31,950	16,425	5,277	280	105	34.25	61.50
Collard	7.78	3.11	4,675	41,500	20,800	6,652	242	247	12.25	68.50
Sesame	10.23	3.04	3,525	20,525	17,400	7,915	660	170	15.00	77.50
Sorghum	11.17	1.45	2,557	25,475	3,830	3,772	175	110	9.50	56.75
Velvetbean	8.68	2.20	2,255	13,925	14,025	3,905	460	1070	12.00	60.75
Zinnia	10.19	2.30	2,910	32,075	14,275	9,437	710	125	8.75	53.00

stage juveniles (J2) was determined from a 100-cm^3 sample from the infested soil by the centrifugal flotation technique (Jenkins, 1964). The control soil sample was also sampled to certify absence of the *M. arenaria* race 1 by the same technique. The composition of the soil mixture for both experiments was 95% sand, 4% clay, and 1% silt.

For both experiments, the nematode-susceptible 'Clemson Spineless' okra was planted in pots in the greenhouse. In the first experiment, the amendments were applied to okra grown from seeds, and in the second experiment, transplanted okra was used. Other differences between the two experiments, mainly in the amounts of amendments used and sampling dates, are described below.

In both experiments, the experimental design was a 6 x 2 x 2 factorial with six organic amendments, two forms of organic amendments (fresh and dry), and two nematode densities, replicated six times. Additional control pots (without mulch) were introduced to verify the okra growth under greenhouse conditions with the presence or absence of *M. arenaria* race 1. These were not included in the statistical analysis, since the objective of the experiments was to verify if there were differences among mulch types regarding nematode dynamics and plant performance. In addition, it was known from previous trials that there was a beneficial effect on plant growth from use of mulch under inoculum or no inoculum treatments (Chapters 2, 3 and 4).

Plants in both experiments were watered as needed and sprayed two times per week with dilute soap solution to reduce infestations of whiteflies (*Bemisia tabaci*). The greenhouse temperature and soil moisture were monitored throughout the experiments.

At harvest, plants were removed from the soil, and shoot and taproot lengths as well as the fresh and dry weights were recorded. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0= 0 galls or egg masses per root system, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100, and 5= more than 100 galls or egg masses per root system.

The final numbers of second-stage juveniles hatching from egg masses in the okra root system were quantified by extracting eggs in 1.05% NaOCl (Hussey and Barker, 1973), then incubating the eggs on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days, and then counting the hatched J2. Population levels of J2 in soil were quantified from 100 cm^3 of soil by the centrifugal flotation technique (Jenkins, 1964).

Plant data from each experiment were statistically analyzed as a completely randomized 6 x 2 x 2 factorial, with six replications, to determine the main effects and the interactions between each two factors and among the three factors. Where appropriate, means were separated by Tukey's test (SAS Institute, Cary, NC). Nematode data were analysed as a 6 x 2 factorial.

When a main effect was significant with no interactions, a separate analysis was carried out. The least-squares means procedure (LSMEANS procedure of SAS Institute, Cary, NC) was used to summarize the effects when the numbers of replications varied (due to dead plants, lack of germination, etc.) throughout the study. Data on galls and egg masses were analyzed from six replications, but data on juveniles hatched from the egg masses or juveniles extracted from 100 cm^3 of soil were analyzed from only three replications.

Experiment 1 (Winter 1995)

Okra in this experiment was grown from seed in plastic pots filled with 380 cm^3 of soil (600 g dry weight of soil). Initial nematode numbers were 120 J2/pot in the infested soil treatment and 0 J2/pot in the non-inoculated treatment. On 17 December 1994, two okra seeds were planted one cm deep in each pot. After plant emergence, the seedlings were thinned to one, on 29 December 1995.

The mulch treatment consisted of 4 g of dry or fresh weight of each organic amendment. Fresh mulch was made by chopping the aerial parts of each plant species into small pieces and immediately weighing 4 g of each one. Dry mulch was proceded by drying the chopped aerial parts until constant weight was reached and then weighing 4 g of each one. The mulch was placed individually at the top of the pot, at the time when seeds were sown. Plant height and stem diameter were recorded 42 days after planting. Plants were harvested at 65 days after planting (20 February 1995). Nematode evaluations consisted of egg masses and galls ratings, and counting juveniles hatched from the egg masses or extracted from the soil. Minimum temperatures during the experimental period ranged from 4.2 to 8.4 ^{0}C, and maximum temperatures ranged from 18.6 to 20.4 ^{0}C.

Experiment 2 (Summer 1995)

For this experiment, okra seeds were individually sown in Speedling^{TM} trays (27 cm x 52 cm, with capacity for 36 seedlings) in a growth room. These trays were filled

with steam-sterilized sandy soil (92% sand, 4% silt, 4% clay), kept at 24 ± 1 °C, and watered daily. After two weeks, on 27 May 1995, these seedlings were individually transplanted into pots, filled with 560 cm^3 of soil (1.2 kg dry weight). Pots were watered to field capacity immediately after transplanting. On 27 May, it was verified that the infested pot treatments had only 28 J2 per pot, therefore these pots were inoculated with an additional 140 J2 per pot, totalling 168 J2 per infested pot. Non-infested pots had 0 J2 per pot. The dry mulch treatment consisted of 4 g of each organic amendment previously dried to constant weight, according to the same procedure used in experiment 1. However, the fresh weight was the corresponding amount needed to get 4 g dry weight. These amounts were 32.14 g, 13.55 g, 26.16 g, 27.60 g, 31.26 g, and 17.58 g for sesame, sorghum, collard, castor, zinnia, and velvetbean, respectively.

Data on plant height and stem diameter were taken on 23 June, 30 days after transplanting. On 9 July, 42 days after transplanting, plants were harvested and the following data were recorded for each replication: shoot and taproot length, fresh and dry weights of shoot and root, ratings of root galls and egg masses, juveniles from egg masses and 100 cm^3 of soil. Minimum temperatures during the experimental period ranged from 18.0 to 21.6 ^{0}C, and maximum temperatures ranged from 31.7 to 33.5 ^{0}C.

Results and Discussion

When the susceptible 'Clemson Spineless' okra was grown during the winter season in the first experiment, the temperature was relatively low for this plant. Consequently, there were no significant differences between plants grown in pots without

organic amendment in the presence or absence of nematodes for most of the plant parameters (Table 5.2). There was infection by the root-knot nematode in the root system. However, nematode population growth was slower than would be expected under more favorable temperatures; considering that 65 days could be enough for the second nematode generation to occur, the final nematode population (Pf) was expected to be much higher than the Pf observed (Davide and Triantaphylllou, 1967; Roberts et al., 1981).

Fresh or dry root weights were higher in the presence of nematode due to the presence of swollen roots infected by the root-knot nematode. The same result was observed in the second experiment, which was carried out in the summer. However, the second experiment had to be harvested at 45 days because of other factors which could have influenced the results, such as size of the pots, and the reproductive stage of the plants. Nematode population growth in the second experiment was faster than in the first experiment since the final nematode population was about seven times bigger than the initial nematode population (Table 5.2). Gall and egg masses, as well as juveniles extracted from the root system or soil samples were higher in the second experiment (Table 5.2).

Nonetheless, for both experiments, plants grown in pots without an organic amendment but in the presence of *M. arenaria* were infected and had high numbers of galls and egg masses, which validated the treatments responses under different mulch types.

Table 5.2. Growth of okra under greenhouse condition in Experiment 1 (Winter 1995) and Experiment 2 (Summer 1995). Responses under inoculum and no inoculum with second-stage juveniles (J2) of *Meloidogyne .arenaria* race 1. Data are averages of six pots without mulch.

Control pots (no mulch)	Plant height (cm)		Stem diam. (mm)	Taproot length (cm)	Root weight (g)		Top weight (g)		J2/root system	J2/100 cm^3 soil	J2/g root	Egg [††] masses	Gall [††] index
	Early[†]	Harvest			Fresh	Dry	Fresh	Dry					
Experiment 1 - Winter 1995 [§]													
0 J2/pot	16.7 a	15.2 a	2.6 b	12.2 a	0.9 b	0.1 b	1.8 a	0.4 a					
120 J2/pot	15.1 a	13.3 a	3.1 a	14.0 a	2.0 a	0.3 a	1.5 a	0.2 a	79.3	25	310.6	4.3	5.0
Experiment 2 - Summer 1995 [§]													
0 J2/pot	46.2 a	60.3 a	4.8 a	23.0 a	4.3 b	0.7 b	15.4 a	3.7 a					
168 J2/pot	43.3 a	53.7 b	4.9 a	24.5 a	9.7 a	1.6 a	13.0 b	2.9 b	173.0	178.0	104.4	4.8	5.0

[†] Measured at 42 days in experiment 1 and 30 days at experiment 2.

[††] Egg masses and galls rated on 0 to 5 scale, where 0= no egg mass or gall; 1= 1 to 2; 2= 3 to 10; 3= 11 to 30; 4= 31 to 100; and 5= more than 100 egg masses or galls per root system.

[§] Within each experiment, means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according Tukey's test.

Experiment 1

There was no triple interaction ($P \geq 0.05$) for any of the plant parameters assessed (Table 5.3). Nevertheless, there was a significant interaction ($P \leq 0.01$) between mulch type and mulch form (fresh or dry organic amendment) for all plant parameters except taproot length (Table 5.3). For stem diameter, plant height, root weight, and top weight, the higher value was generally recorded when the organic amendment was in the dry form. Using dry velvetbean, castor, collard, and zinnia generally resulted in higher plant responses than sesame or sorghum (Table 5.4). However, under fresh organic amendment there was no specific trend for mulch type.

The inoculum level x mulch type interaction was significant ($P \leq 0.05$) only for top dry weight (Table 5.3). Amendments that had low C/N ratio (velvetbean, castor, collard, and zinnia) applied at the no inoculum level had higher values for top dry weight (data not shown).

Significant differences ($P \leq 0.01$) were found for inoculum level in all plant parameters assessed (Table 5.3). The highest values generally were reached at 0 J2/pot, except for root length, root fresh, or dry weight (data not shown). The higher values in the nematode-infected roots were due to the presence of swollen roots and galls, which was confirmed by the control treatment (Table 5.2). Plant growth responses were consistently higher with dry amendments and for those amendments that had a low C/N ratio (Table 5.4).

In Experiment 1, there was a significant interaction ($P \leq 0.01$) between mulch form and mulch type for juveniles hatched from egg masses either per root system or per gram

Table 5.3. Effect of inoculum level, mulch form (fresh or dry), and mulch type on plant parameters of 'Clemson Spineless' okra in greenhouse experiments. Data shown are F-values from the analysis of variance.

Treatment effect	Stem diameter	Plant height		Taproot length	Root weight		Top weight	
		Early†	Harvest		Fresh	Dry	Fresh	Dry
Experiment 1 - Winter 1995								
Nematode inoc. (A)	41.79**	17.12**	19.09**	9.90**	152.41**	123.20**	9.74**	32.32**
Mulch form†† (B)	1.44 ns	10.49**	29.30**	0.05 ns	15.11**	5.72*	73.12**	47.26**
Mulch type (C)	13.03**	5.29**	8.13**	1.60 ns	39.05**	26.38**	27.24**	20.52**
Interaction (AB)	1.41 ns	0.33 ns	2.13 ns	0.72 ns	0.24 ns	0.46 ns	0.24 ns	0.06 ns
Interaction (AC)	2.11 ns	2.22 ns	1.85 ns	2.24 ns	0.86 ns	0.83 ns	1.29 ns	2.35*
Interaction (BC)	5.88**	3.75**	6.17**	1.89 ns	18.33**	14.37**	16.60**	13.35**
Interaction (ABC)	0.48 ns	1.54 ns	1.06 ns	0.48 ns	0.95 ns	1.14 ns	0.72 ns	0.50 ns
Experiment 2 - Summer 1995								
Nematode inoc. (A)	81.53**	125.74**	174.83**	12.96*	422.66**	386.28**	138.50**	178.36**
Mulch form†† (B)	0.35 ns	1.23 ns	4.82*	0.01 ns	3.58 ns	16.43**	5.86*	0.37 ns
Mulch type (C)	18.81**	3.26**	5.05**	1.73 ns	21.93**	21.85 ns	27.69**	16.16**
Interaction (AB)	3.50 ns	2.50 ns	3.74 ns	1.52 ns	0.21 ns	3.71 ns	1.58 ns	2.66 ns
Interaction (AC)	3.49**	1.25 ns	2.01 ns	0.54 ns	3.92*	3.65**	2.80*	0.51 ns
Interaction (BC)	1.58 ns	0.53 ns	0.55 ns	1.38 ns	2.18 ns	2.06 ns	1.84 ns	1.85 ns
Interaction (ABC)	2.05 ns	0.93 ns	1.74 ns	1.93 ns	1.28 ns	1.53 ns	1.66 ns	0.87 ns

*, ** Indicate significant main effect or interaction at P≤ 0.05 and P≤ 0.01, respectively; ns= effect not significant at P≤ 0.05.

† Measured at 42 days in experiment 1 and 30 days at experiment 2.

†† Fresh or dry mulch.

Table 5.4. Effect of mulch type and mulch form (fresh or dry) on plant growth, Experiment 1 (Winter 1995).

Mulch type	Stem diameter (mm)			Plant height (cm)						Root (g) fresh weight		
				42 days			65 days					
	Fresh	Dry	Mean	Fresh	Dry	Mean	Fresh	Dry	Mean	Fresh	Dry	Mean
Castor	3.4 a	3.4 a	3.4	15.7 a	18.3ab	17.0	14.4 a	18.2ab	16.3	1.7 a	2.1 b	1.9
Collard	3.1 a	3.7 a	3.4	15.6 a	18.1ab	16.8	14.8 a	16.4bc	15.6	1.6 a	2.1 b	1.8
Sesame	3.1 a	2.6 b	2.8	15.7 a	16.1bc	15.9	14.4 a	15.8bc	15.1	1.5 a	1.2 c	1.3
Sorghum	2.9 a	2.6 b	2.7	15.1 a	13.9 c	14.5	14.3 a	14.1 c	14.2	1.4 a	1.1 c	1.2
Velvetbean	3.3 a	3.6 a	3.4	15.9 a	19.8 a	17.8	15.0 a	21.1 a	18.0	1.8 a	3.1 a	2.4
Zinnia	2.9 a	3.3 a	3.1	16.3 a	16.3abc	16.3	15.0 a	15.9bc	15.4	1.5 a	1.4 bc	1.4
Mean	3.1	3.2	3.1	15.7	17.1	16.3	14.6	16.9	15.7	1.6	1.8	1.7

Data are means of six replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according Tukey's test.

Table 5.4. (continued ...)

Mulch type	Root dry weight (g)			Top fresh weight (g)			Top dry weight (g)		
	Fresh	Dry	Mean	Fresh	Dry	Mean	Fresh	Dry	Mean
Castor	0.2 a	0.3 ab	0.28	2.4 a	3.5 b	2.9	0.4 a	0.6 b	0.5
Collard	0.2 a	0.3 ab	0.26	2.1 a	3.7 b	2.9	0.4 a	0.6 b	0.5
Sesame	0.2 a	0.1 d	0.17	2.0 a	2.2 cd	2.1	0.4 a	0.4 cd	0.4
Sorghum	0.2 a	0.1 d	0.17	1.9 a	1.5 d	1.7	0.3 a	0.3 d	0.3
Velvetbean	0.2 a	0.4 a	0.32	2.3 a	5.0 a	3.7	0.4 a	0.9 a	0.7
Zinnia	0.2 a	0.2 bc	0.21	2.0 a	3.0 bc	2.5	0.4 a	0.6 bc	0.5
Mean	0.22	0.25	0.23	2.1	3.2	0.39	0.39	0.57	0.57

Data are means of six replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according Tukey's test.

dry weight of the root system (Table 5.5). However, none of the factors affected (P≥0.05) juveniles extracted from soil (Table 5.5). Probably, the greenhouse temperature (data not shown) was not favorable for the development of this second generation of *M. arenaria*. This hypothesis is based on data from the control pots and is in accordance with findings of Davide and Triantaphyllou (1967). Generally, with dry organic amendments, the lowest number of J2 hatched from egg masses were recorded under castor, collard,

Table 5.5. Effect of mulch form (fresh or dry), and mulch type on *Meloidogyne arenaria* infecting 'Clemson Spineless' okra. Data shown are F-values from the analysis of variance.

Treatment effect	Juveniles (J2)			Gall index‡	Egg mass index‡
	(J2)/root system†	(J2)/g root†	(J2)/100 cm^3 soil§		
	Experiment 1 - Winter 1995				
Mulch form (A)	27.28**	0.96 ns	0.02 ns	15.72**	14.30**
Mulch type (B)	33.26**	78.09**	0.38 ns	2.36 ns	3.73**
Interaction (AB)	19.69**	57.76**	1.24 ns	0.95 ns	0.69 ns
	Experiment 2 - Summer 1995				
Mulch form (A)	0.93 ns	3.15 ns	3.57 ns	0.00 ns	3.00 ns
Mulch type (B)	2.61 ns	3.78*	3.73**	2.00 ns	10.00**
Interaction (AB)	0.27 ns	0.27 ns	0.65 ns	0.00 ns	0.55 ns

*, ** Indicate significant main effect or interaction at P≤ 0.05 and P≤ 0.01, respectively; ns= effect not significant at P≤ 0.05.

† Data are based on three replications.

†† Data are based on six replications.

§ Data are average of 3 replications for experiment 1, and 6 for experiment 2.

velvetbean, and zinnia, which have low C/N ratios. The highest numbers were recorded under sesame and sorghum, which have higher C/N ratios (Table 5.6). However, with

Table 5.6. Effect of mulch type and mulch form (fresh or dry) on numbers of second-stage juveniles (J2) of *Meloidogyne arenaria*, and on gall and egg mass indices, Experiment 1 (Winter 1995).

Mulch	J2/g root			J2/root system		
type	Fresh	Dry	Mean	Fresh	Dry	Mean
Castor	198.4 bc	98.2 b	148.3	64.0 bc	37.3 cd	50.7
Collard	174.5 c	103.8 b	139.1	45.0 c	42.3 bc	43.7
Sesame	145.0 c	478.3 a	311.7	41.0 c	71.7 ab	56.3
Sorghum	375.1 a	438.8 a	406.9	106.7 a	83.0 a	94.8
Velvetbean	197.1 bc	123.7 b	160.4	60.3 bc	55.0 bc	57.7
Zinnia	277.3 b	64.4 b	170.8	73.0 b	16.7 d	44.8
Mean	227.9	217.9	225.3	65.0	51.0	58.0

† Egg masses and galls rated on 0 to 5 scale, where 0 = no egg mass or gall; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 egg masses or galls per root system.

Data are means of six replications. Means followed by the same letter in columns are not significantly different (P≤0.05), according to Tukey's test.

Table 5.6. (continued ...)

Mulch	Root gall index[†]			Egg mass index[†]		
type	Fresh	Dry	Mean	Fresh	Dry	Mean
Castor	4.83	4.20	4.51 b	3.67	3.00	3.3 b
Collard	5.00	4.80	4.90 ab	4.00	3.60	3.8 ab
Sesame	5.00	4.83	4.92 a	4.50	4.00	4.2 a
Sorghum	5.00	4.50	4.75 ab	4.17	4.00	4.1 a
Velvetbean	5.00	4.83	4.92 a	4.00	3.67	3.8 ab
Zinnia	5.00	4.67	4.83 ab	4.33	3.33	3.8 ab
Mean	4.97	4.64	4.8	4.11 A	3.60 B	3.8

[†] Egg masses and galls rated on 0 to 5 scale, where 0 = no egg mass or gall; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 egg masses or galls per root system.
Data are means of six replications. Means followed by the same letter in columns are not significantly different (P≤0.05), according to Tukey's test.

this trend was not observed with fresh amendments, although the highest number of hatched J2 was observed under sorghum amendment (Table 5.6).

Formation of galls and egg masses by *M. arenaria* was affected ($P \leq 0.01$) by the organic amendment form (fresh or dry) and by the organic amendment type (Table 5.5). For both indices, the higher value was recorded with the fresh organic amendment and the lower value with the dry organic amendment (Table 5.6). The reason for these results lies in the fact that for each amendment, either 4 g of fresh weight or 4 g of dry weight was used, so more active amendment (less water) was actually applied in the dry amendment treatment.

In general, the significant effect of mulch type treatment or the interaction between mulch type and mulch form revealed highest values for plant parameter responses and effective suppression of nematodes under castor, collard, velvetbean, or zinnia amendments in the dry form. These amendments tended to have the lowest C/N ratios (Table 5.1). There were no similar trends among mulch types with fresh organic amendments (Table 5.4). Data on the macro- or micronutrients in soil (Table 5.1) did not provide much evidence that any of these may have contributed to the response of the plant parameters or nematode dynamics. It was evident, however, that the mulches themselves had beneficial effects on the plants, regardless of inoculum treatments. These results are in accordance with findings in previous experiments (Chapters 2, 3, and 4).

Experiment 2

The effects of mulch type were significant ($P \leq 0.01$) for almost all plant parameters, except taproot length and root dry weight (Table 5.3). The highest values of most plant parameters were generally obtained from velvetbean, castor, collard, or zinnia treatments, and the lowest values from sesame or sorghum. These results are in accordance with findings reported in the first experiment.

There was no interaction ($P \geq 0.05$) between mulch form and mulch type, which suggests that the equivalent weight of fresh or dry amendment had the same effect on plant parameters (Table 5.3) and nematode suppression (Table 5.5). The effects of mulch form were significant only for root dry weight ($P \leq 0.05$), plant height at harvest ($P \leq 0.01$), and top fresh weight ($P \leq 0.05$). However, there were significant interactions between inoculum level and mulch type for stem diameter ($P \leq 0.01$), top fresh weight ($P \leq 0.05$), root fresh weight ($P \leq 0.05$), and root dry weight ($P \leq 0.01$) (Table 5.3). Inoculum level significantly affected ($P \leq 0.01$) all plant parameters (Table 5.3). The highest responses for plant height, stem diameter, and top weight were obtained with no inoculum under castor, collard, velvetbean, or zinnia amendments (Table 5.7). Greater taproot lengths (data not shown) and root weights (Table 5.7), were obtained in the presence of *M. arenaria*. Inoculation resulted in higher values due to infection by the nematode, which caused swollen roots and galls. These results were in accordance with the findings in the first experiment.

There were no significant interactions between mulch type and mulch form for any of the nematode evaluations (Table 5.5). Nonetheless, mulch type affected J2/g root

Table 5.7. Effect of mulch type and *Meloidogyne arenaria* inoculum level (J2/pot) on plant parameters, Experiment 2 (Summer 1995).

Mulch	Root fresh weight (g)			Root dry weight (g)		
Type	0 J2/pot	168 J2/pot[†]	Mean	0 J2/pot	168 J2/pot	Mean
Castor	7.20 ab	11.72 b	9.46	1.04 ab	1.68 ab	1.36
Collard	7.83 a	13.45 a	10.64	1.21 a	2.09 a	1.65
Sesame	5.42 c	9.97 cd	7.69	0.85 b	1.57 bc	1.20
Sorghum	5.75 bc	9.10 d	7.42	0.83 b	1.32 c	1.08
Velvetbean	7.02 abc	9.85 cd	8.43	1.14 a	1.61 b	1.38
Zinnia	6.37 abc	11.04 bc	8.70	0.99 ab	1.73 b	1.36
Mean	6.60	10.8	8.72	1.01	1.67	1.33

[†] Number of juveniles (J2) hatched from the egg masses and used as inoculum.

Data are means of six replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according to Tukey's test.

Table 5.7. (continued ...)

Mulch	Stem diameter (mm)			Top fresh weight (g)		
Type	0 J2/pot	168 J2/pot†	Mean	0 J2/pot	168 J2/pot	Mean
Castor	5.55 bc	5.28 ab	5.4	25.32 ab	20.04 ab	22.68
Collard	5.79 ab	5.61 a	5.7	26.47 ab	23.19 a	24.83
Sesame	5.32 c	5.05 bc	5.2	21.03 c	16.91 bc	18.97
Sorghum	5.35 c	4.75 c	5.0	20.65 c	13.95 c	17.30
Velvetbean	6.02 a	5.30 ab	5.6	28.82 a	20.18 ab	24.50
Zinnia	5.69 abc	5.13 bc	5.4	24.18 b	19.16 b	21.67
Mean	5.62	5.19	5.30	24.4	18.9	21.6

† Number of juveniles (J2) hatched from the egg masses and used as inoculum.
Data are means of six replications. Means followed by the same letter in columns are not significantly different ($P \leq 0.05$), according to Tukey's test.

Table 5.7. (continued ...)

Mulch Type	Plant height (cm)						Top dry weight (g)		
	30 days			45 days					
	0 J2/pot	168 J2/pot†	Mean	0 J2/pot	168 J2/pot†	Mean	0 J2/pot	168 J2/pot	Mean
Castor	52.1 a	43.6 a	47.0 ab	70.7 a	60.1 abc	65.0 ab	5.51 ab	4.05 ab	4.78 ab
Collard	50.7 a	45.4 a	48.0 ab	68.8 a	61.2 ab	65.0 ab	5.78 a	4.44 a	5.11 a
Sesame	49.1 a	43.6 a	46.3 b	66.7 a	57.6 abc	62.0 b	4.76 b	3.22 bc	3.99 cd
Sorghum	51.5 a	42.4 a	46.9 ab	68.9 a	54.2 c	61.0 b	4.71 b	2.79 c	3.75 d
Velvetbean	52.8 a	47.1 a	50.0 a	71.2 a	62.7 a	67.0 a	5.98 a	4.30 a	5.14 a
Zinnia	49.7 a	43.5 a	46.6 b	68.0 a	59.4 abc	64.0 ab	5.14 ab	3.63 ab	4.39 bc
Mean	51.0 A	44.3 B	47.6	69.0 A	59.2 B	64.0	5.31 A	3.74 B	4.52

† Number of juveniles (J2) hatched from the egg masses and used as inoculum.

Data are means of six replications. Means followed by the same letter in columns are not significantly different (P≤0.05), according to Tukey's test.

($P \leq 0.05$), egg masses ($P \leq 0.01$), and J2/100 cm^3 soil ($P \leq 0.01$) (Table 5.5). Generally, sesame and sorghum amendments resulted in the highest values for these parameters and castor, collard, velvetbean, or zinnia the least (Table 5.8).

Table 5.8. Effect of main effect of mulch type on numbers of *Meloidogyne arenaria* juveniles (J2) in soil and roots and on egg mass and gall indices. Experiment 2 (Summer 1995).

Mulch Type	J2/g root	J2/100 cm^3 soil	Egg mass index[†]	Root gall index
Castor	120.7 ab	24.92 b	3.83 b	5.00 a
Collard	84.8 ab	74.58 ab	4.08 b	5.00 a
Sesame	171.7 a	133.33 ab	4.67 a	5.00 a
Sorghum	138.0 ab	174.25 a	4.75 a	5.00 a
Velvetbean	44.9 b	11.83 b	4.00 b	4.83 a
Zinnia	94.4 ab	82.17 ab	4.17 b	5.00 a

[†] Egg masses and galls rated on 0 to 5 scale, where 0= no egg mass or gall; 1= 1 to 2; 2= 3 to 10; 3= 11 to 30; 4= 31 to 100; and 5= more than 100 egg masses or galls per root system.
Data are means of six replications. Means folowed by the same letter in columns are not significantly different ($P \leq 0.05$), according to Tukey's test.

When J2 were hatched from egg masses/g root system, the lowest number was obtained under velvetbean ($P \leq 0.05$). When J2 were extracted from soil, the lowest numbers were observed under castor and velvetbean and the highest under sorghum. Thus, it was evident that the beneficial effects of the organic amendments were dependent upon the type of the amendment used and should not be generalized. However, the amendments with the lowest C/N ratios were generally better for improvement of plant parameters or suppression of nematodes. These results were in accordance with those obtained from the first experiment.

The significant effect of fresh or dry organic amendment in the first experiment was evident because the 4 g of fresh weight did not represent the corresponding amount of dry weight for all amendments. This was evident because in the second experiment, fresh and dry weights used had proportional values for each amendment, and therefore, there were no significant differences between effects of fresh or dry organic amendment for most plant parameters and for all nematode responses. Thus, it can be concluded that there is no significant difference in the ability of the organic amendment form (fresh vs. dry) to reduce J2 in the root system or in the soil. In general, for both experiments, castor and velvetbean suppressed the nematode population best, followed by zinnia and collard. Plant growth responses were better under castor, velvetbean, collard, or zinnia amendments, which had the lowest C/N ratios. Sesame or sorghum amendments, which had the highest C/N ratios, had much less effect on plant growth and on suppression of nematodes.

CHAPTER 6

EFFECT OF CASTOR AND VELVETBEAN ORGANIC AMENDMENTS ON *MELOIDOGYNE ARENARIA* IN GREENHOUSE EXPERIMENTS

Introduction

A number of organic amendments have been used for management of nematodes, because they are associated with reduced infection or survival of the nematode species, or because they may increase microbial and animal species antagonistic to nematodes (Linford et al., 1938; Watson, 1945; Mankau and Minteer, 1962; Sitaramaiah and Singh, 1978; Mankau, 1968; Muller and Gooch, 1982; Ichinohe, 1985; Trivedi and Barker, 1986; Rodríguez-Kábana, 1986). Of the amendments tested in our experiments, castor (*Ricinus communis* L.) and velvetbean (*Mucuna deeringuiana* [Bort.] Merr.) provided the best results in improving plant growth and reducing nematodes (Chapter 5). There was insufficient evidence to conclude if suppression was due to the release of toxic substances, to improved plant nutrition, or to the enhanced growth of antagonistic organisms. Nonetheless, effectiveness of nematode suppression by using organic amendments still depends on their C/N ratio, amount of the organic amendment used, and time of decomposition (McSorley and Gallaher, 1995a, b).

Singh and Sitaramaiah (1994) reported that the decomposition rate of an amendment depends on the soil type and the climate. Of course, the rate of organic amendment decomposition is related to the amount of the amendment applied, so that

nematode suppression is related to both the quantity and quality of the decomposition products (Singh and Sitaramaiah, 1994). Although many studies have shown that the amount of the organic amendment is important in nematode suppression (Linford et al., 1938; Holtz and Vandecaveye, 1938; Watson, 1945; Mankau and Minteer, 1962; Mankau, 1968; Sitaramaiah and Singh, 1978; Rodríguez-Kábana, 1986; McSorley and Gallaher, 1995b), further research on the quality and quantity of amendments is still needed to stimulate their widespread use (McSorley and Gallaher, 1995a, b).

Castor and velvetbean were chosen for greenhouse studies, because they had been used in earlier tests as organic amendments and had suppressed *Meloidogyne arenaria* (Neal) Chitwood race 1 and provided the best plant growth response (Chapter 5). The objectives of these greenhouse experiments were to compare the effects of these two amendments by determining which one was more effective in improving plant growth and suppressing *Meloidogyne arenaria* race 1 on okra (*Hibiscus esculentus* L.), and to determine the effects of various application rates of these amendments on nematode levels and plant growth.

Materials and Methods

Three separate experiments were carried out in a greenhouse during the summer and fall of 1996, on the University of Florida campus in Gainesville, Florida. Castor and velvetbean plants were grown in 18-cm-diameter clay pots to be used as dry organic amendments for all three experiments (same procedure described in Chapter 5). No fertilizer was applied. Above-ground parts of both types of plants were harvested before

the reproductive stage. Leaves and stems of each one were chopped separately, mixed well and then dried until constant weight was reached. These materials were used as amendments at different rates, according to each experiment, and 6.9 g and 4.4 g of fresh castor and velvetbean were used to obtain 1 g of dry amendment, respectively. A sample of each amendment was analyzed for mineral and C/N ratio, according to the methodology described in Chapter 2 (Gallaher et al., 1975; Walkley and Black, 1934; Walkley, 1947; Mehlich, 1953), and analyses are presented in Table 6.1.

For all experiments, the nematode-susceptible 'Clemson Spineless' okra was used as a test plant. Okra seeds were individually sown in Speedling^{TM} trays (27 cm x 52 cm, with capacity for 36 seedlings) in a growth room. These trays were filled with steam-sterilized sandy soil, consisting of 92% sand, 4% silt, 4% clay, and kept at 24 ± 1 ^{0}C and watered daily. After two weeks, the seedlings were transplanted into pots and served as experimental units for each experiment.

All plants were set in plastic pots filled with 560 cm^3 of steam-sterilized field soil mixed with sand, in a ratio of 1:1 by volume. The composition of the soil mixture for the experiments was 95% sand, 4% clay, and 1% silt. The dry soil weight for each pot was 900 g. After transplanting a two-week-old okra seedling and watering, each pot weighed 1,110 g.

One day after transplanting, the pots received nematode inoculum and the amendment treatments according to the requirement of each experiment. The inoculum treatment consisted of 1,000 fresh second-stage juveniles (J2) of *Meloidogyne arenaria* race 1, which had been increased on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.).

Table 6.1. Plant mineral analysis and C/N ratio from the organic amendment treatments. Data are means of five replications, from the aerial plant parts harvested before the reproductive stage.

Organic	C/N	Macronutrients					Micronutrients			
amendment	ratio	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
		--- % --	----------	----------	----------	----------	ppm	----------	----------	----------
Castor	7.91	2.26	3,400	31,950	16,425	5,277	280	105	34.25	61.50
Velvetbean	8.68	2.20	2,255	13,925	14,025	3,905	460	1070	12.00	60.75

Pots with 0 J2 constituted the no-inoculum treatment. Amendment treatments are specified below for each experiment.

Plants were maintained in a greenhouse. Soil temperature was monitored throughout all experiments. The average minimum and maximum soil temperatures are presented in Table 6.2. Plants were sprayed twice a week with a dilute soap solution to reduce infestations of whiteflies (*Bemisia tabaci*), but otherwise, no pesticides or fertilizers were applied.

Additional control pots (without amendment) were introduced to verify okra growth under greenhouse conditions in the presence or absence of *Meloidogyne arenaria* race 1. For the two first experiments, those data were included in the statistical analysis to trace the regression line (zero amendment points). For the third experiment, these data were not included in the statistical analysis since the objective was to verify the difference between the two amendments. The beneficial use of amendments in improving plant performance was confirmed in all cases.

Data were collected at two different times, which are specified in each experiment. However, data were taken using the same procedures to insure consistency. For instance, at harvest, plants were removed from the soil, and shoot and taproot lengths as well as fresh and dry weights, and stem diameter were recorded. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0 = 0 galls or egg masses per root system, 1 = 1 -2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = more than 100 galls or egg masses per root system.

Table 6.2. Average maximum and minimum soil temperatures (^{0}C) in greenhouse experiments. University of Florida campus, Gainesville, Florida. 1996.

Soil temperature	May	June	July	August	September	October	November
Minimum	21.0 ± 0.8	20.9 ± 1.3	22.7 ± 0.8	22.3 ± 0.7	20.0 ± 1.8	17.9 ± 2.9	17.0 ± 3.0
Maximum	33.0 ± 2.9	33.7 ± 2.5	36.3 ± 2.8	33.3 ± 3.0	35.1 ± 1.1	28.9 ± 2.3	29.6 ± 1.7

For the first and second experiments, the final second-stage juveniles hatching from egg masses in the okra root systems were quantified by extraction in 1.05% NaOCl (Hussey and Barker, 1973). The extracted eggs were then incubated on Baermann trays (Rodríguez-Kábana and Pope, 1981) for seven days and the hatched J2 were counted. For the third experiment, the same technique was used, but with only 10 egg masses from each root system. These egg masses were removed from the root system and put in a small tube, which was then filled with 5 ml of 1.05% NaOCl and vigorously shaken. Contents were then poured onto a 500-mesh screen to collect eggs, which were washed, and then incubated on Baermann trays for four days. After those 10 egg masses were removed from the root system, the root system was immersed in a Phloxine B solution (0.15g/l tap water) for rating all the remaining egg masses and galls by this staining technique (Southey, 1982).

At harvest, a soil sample was taken from each treatment to determine the final nematode population. Second-stage juveniles were determined from 100 cm^3 soil from the infested treatments using the centrifugal flotation technique (Jenkins, 1964). Soil samples from control treatments were also examined to certify absence of *M. arenaria* race 1, using the same technique.

The experimental design differed somewhat for each experiment. Nonetheless, data were statistically analyzed as a completely randomized factorial in all cases, with five replications in the first and second experiments for each harvest time, and with six replications in the third experiment. In the first two experiments, which involved different rates of amendments, a regression line was traced to determine the pattern of

response of each measured variable to the amendment rate at each inoculum level. Since a quadratic regression resulted in a better fit (higher r^2) for all cases, it was chosen to represent the trend rather than a linear regression. Analysis of variance was applied to the factorial designs, and when a main effect was significant with no interactions, a separate analysis was carried out, and the means were separated by Tukey's test (SAS Institute, Cary, NC).

Experiment 1 (Velvetbean amendment)

On 27 May 1996, two-week-old okra seedlings were transplanted individually into each pot receiving inoculum and amendment rate treatments. The inoculum treatment consisted of 0 J2/pot or 1,000 J2/pot. The amendment was applied as a mulch at the top of the pot, at rates of 0, 1, 2, 4, or 8 g of dry velvetbean per pot. Therefore the experimental design was a 5 x 2 factorial, with 10 replications (5 for each harvest date). Data were analyzed as a completely randomized design followed by regression analysis to determine the response of the okra due to amendment rate under inoculum and no inoculum conditions. On 1 July, 35 days after inoculation, half of the experiment was harvested, and plant parameters and nematode evaluations were recorded, except for J2 from 100 cm^3 soil. Finally, on 23 July, 57 days after inoculation, the remainder of the experiment was harvested and the same plant parameters and nematode evaluations were recorded, as well as J2 extracted from the soil.

Experiment 2 (Castor amendment)

This experiment was similar to the first, but two-week-old okra seedlings were transplanted and inoculated on 18 June 1996. The inoculum consisted of 0 J2/pot or 1,000 J2/pot. The amendment consisted of dry castor applied as a mulch, at rates of 0, 1, 2, 4, or 8 g per pot. The experiment was a 5 x 2 factorial with 10 replications. On 25 July, 37 days after inoculation, half of the experiment was harvested following the same procedure as in the first experiment. On 17 August, 60 days after inoculation the remaining pots of the experiment were harvested. Plant parameters were assessed and nematode evaluations were recorded as described previously.

Experiment 3 (Combined amendments, velvetbean and castor)

On 24 September 1996, two-week-old okra seedlings were inoculated with 0 J2/pot or 1,000 J2/pot. The amendment treatments were castor or velvetbean, both applied at rates of 2 or 8 g per pot. The experimental design was a 2 x 2 x 2 factorial with 12 replications, including two inoculum densities, two amendments, and two amendment rates. Thirty days after inoculation, on 24 October, half of the experiment was harvested, and all the plant parameters and nematode evaluations were recorded, except J2 extracted from soil. On 7 November, 45 days after inoculation, the remainder of the experiment was harvested, and the same plant and nematode evaluations were recorded along with J2 from soil. Additional pots, without amendment, were introduced to verify the okra growth under greenhouse conditions in the presence or absence of *Meloidogyne arenaria* race 1, but these treatments were not included in the statistical analysis.

Results and Discussion

Experiment 1 (Velvetbean amendment)

There were significant interactions between inoculum and amendment rate for stem diameter ($P \leq 0.01$), plant height at 35 days after inoculation ($P \leq 0.01$), and top dry weight at 57 days after inoculation ($P \leq 0.05$) (Table 6.3). The relationships between these plant parameters and amendment rate were fit by quadratic regressions (Table 6.4). In general, these plant parameters increased to a certain point as amendment rate increased, and tended to level off at the highest rate (Figures 6.1, 6.2, 6.3). The quadratic shape was more evident for the inoculated pots, and approached a more linear shape with the non-inoculated pots. An exception was observed for plant height at 35 days after inoculation when plant height under no inoculum decreased as amendment rate increased (Figure 6.4). Under inoculum treatment, plant height response was positively related to the amendment rate, while under no inoculum treatment the response was negative. For the no-inoculum treatment, the r^2 value of a fitted linear regression was of 0.46, compared to the quadratic r^2 of 0.48 (Figure 6.4). It seems that the amendment was beneficial for plant height only under inoculum treatment. Thus, the efficiency of the amendment was limited and was dependent on the inoculum and amendment rate as well as plant age.

There was a significant main effect of amendment rate on plant height ($P \leq 0.01$) at 57 days after inoculation, on top fresh ($P \leq 0.01$) and dry ($P \leq 0.05$) weight at 35 days after inoculation, on top fresh weight ($P \leq 0.01$) at 57 days, on taproot length ($P \leq 0.05$) at 57 days, on root fresh weight ($P \leq 0.01$) at 35 days, on and on root fresh or dry weight ($P \leq 0.01$) at 57 days after inoculation (Table 6.3). These plant parameters had quadratic

Table 6.3. Effect of inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* and velvetbean soil amendment rate (0, 1, 2, 4, or 8 g) on plant parameters of 'Clemson Spineless' okra in a greenhouse experiment. Data shown are F-values from the analysis of variance. Summer 1996.

Treatment	Stem diameter		Plant height		Top weight				Taproot lenght		Root weight			
	35	57	35	57	35 days		57 days		35	57	35 days		57 days	
effect	days	days	days	days	Fresh	Dry	Fresh	Dry	days	days	Fresh	Dry	Fresh	Dry
Inoculum (A)	1.4 ns	6.9*	8.7**	14.7**	3.1 ns	0.03 ns	2.1 ns	18.9**	4.1*	6.2*	55.2**	0 ns	182.0**	18.6**
Rate (B)	15.7**	34.7**	2.9*	9.4**	24.1**	3.2*	44.4**	21.8**	0.6 ns	2.6*	7.0**	1.5 ns	27.2**	19.7**
Interaction AB)	4.9**	5.2**	4.8**	2.5 ns	1.5 ns	1.6 ns	2.1 ns	2.8*	0.8 ns	1.0 ns	0.8 ns	0.5 ns	2.3 ns	2.3 ns

*, ** Indicate significant main effect or interaction at P≤0.05 and P≤0.01, respectively, ns= effect not significant at P≤0.05.

Table 6.4. Effect of velvetbean soil amendment rates and inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* on plant parameters of 'Clemson Spineless' okra. Summer 1996.

Amendment rate	Stem diameter (mm)			
	35 days		57 days	
(g)	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot
0	4.04†	3.62	4.20	3.82
1	3.94	3.90	4.06	4.22
2	4.34	4.54	4.36	4.80
4	4.08	4.68	4.46	4.90
8	4.52	4.62	4.98	5.18
Regression	$Y= 0.004X^2 + 0.02X + 4.0$	$Y= - 0.04X^2 + 0.4X + 3.6$	$Y= 0.006X^2 + 0.06X + 4.1$	$Y= - 0.04X^2 + 0.4X + 3.9$
	$r^2= 0.62$	$r^2= 0.94$	$r^2= 0.94$	$r^2= 0.94$
Significance	$P\leq 0.01$	$P\leq 0.01$	$P\leq 0.01$	$P\leq 0.01$

† Data are means of five replications

Table 6.4. (Continued ...)

Amendment	Plant height (cm) - 35 days		Top dry weight (g) - 57 days	
rate (g)	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot
0	45.6†	37.2	2.77	1.57
1	42.0	41.4	2.73	2.24
2	45.8	43.4	3.11	2.87
4	43.2	43.0	3.20	3.03
8	41.0	41.6	3.85	3.51
Regression	$Y= - 0.04X^2 - 0.2X + 44.6$	$Y= - 0.28X^2 + 2.68X + 38.2$	$Y= 0.003X^2 + 0.11X + 2.7$	$Y= - 0.04X^2 + 0.5X + 1.7$
	$r^2= 0.48$	$r^2= 0.82$	$r^2= 0.96$	$r^2= 0.95$
Significance	P≤0.05	P≤0.05	P≤0.01	P≤0.01

† Data are means of five replications.

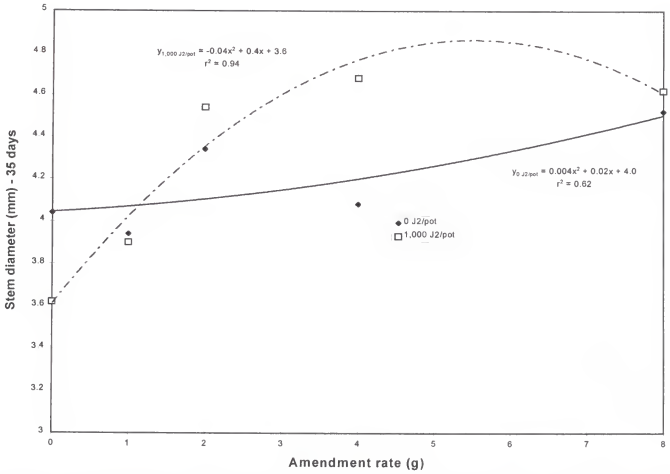

Figure 6.1. Quadratic relationships between okra stem diameter at 35 days and rate of velvetbean amendment, at 0 J2/pot and 1,000 J2/pot. Velvetbean experiment. Points = actual data; lines = fitted regression curve.

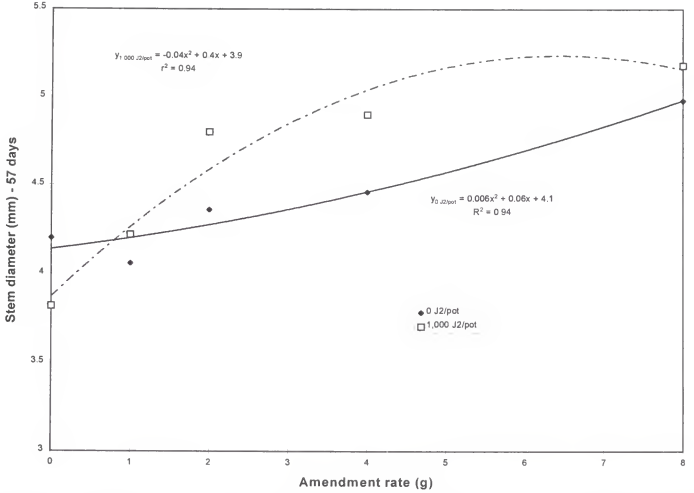

Figure 6.2. Quadratic relationships between okra stem diameter at 57 days and rate of velvetbean amendment, at 0 J2/pot and 1,000 J2/pot. Velvetbean experiment. Points = actual data; lines = fitted regression curve.

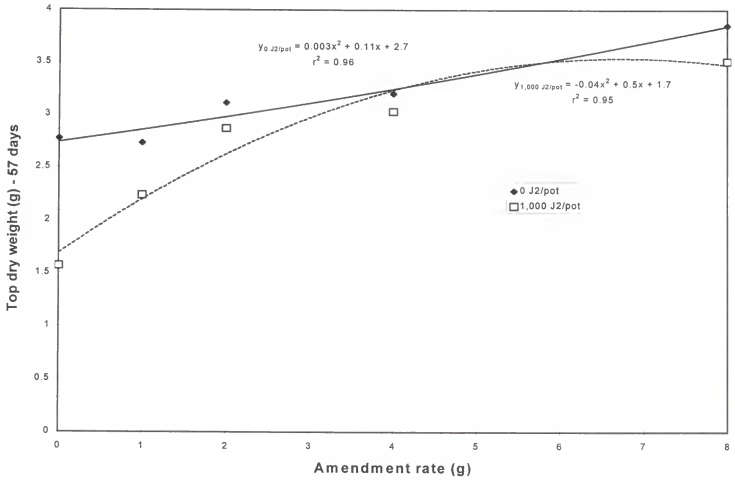

Figure 6.3. Quadratic relationships between okra top dry weight at 57 days and rate of velvetbean amendment, at 0 J2/pot and 1,000 J2/pot. Velvetbean experiment. Points = actual data; lines = fitted regression curve.

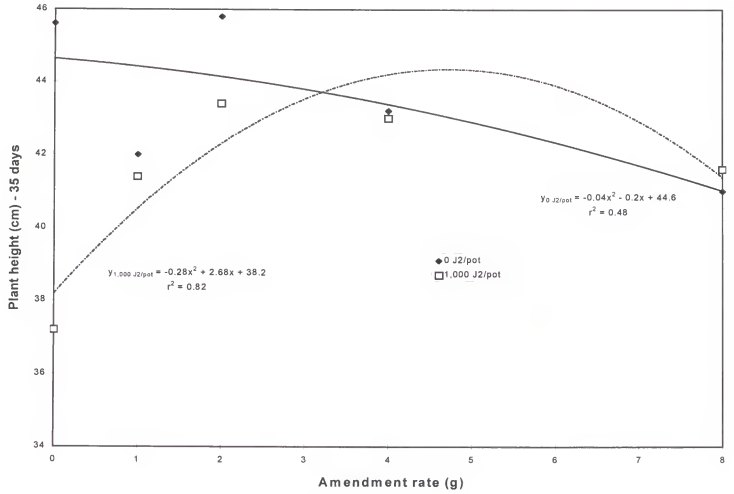

Figure 6.4. Quadratic relationships between okra height at 35 days and rate of velvetbean amendment, at 0 J2/pot and 1,000 J2/pot. Velvetbean experiment. Points = actual data; lines = fitted regression curve.

responses to amendment rate, except for top fresh weight at 57 days, which was better presented by a linear regression (r^2= 0.98) (Table 6.5).

Plant height responded positively to amendment rates up to 4 g, but at 8 g, plant height did not improve further (Figure 6.5). The highest response to the amendment under the quadratic regression (r^2= 0.96) might occur between rates of 5 to 6 g of the amendment (Table 6.5; Figure 6.5). Top weight at 35 days had a similar quadratic trend (Table 6.5). Generally, top weight increased until a certain level of the amendment, but after that, other factors like toxicity of the amendment may have inhibited plant response. However, fresh top weight at 57 days was better fitted by a linear regression (r^2= 0.98), which implies that as amendment rate increases, plant height will also increase, at least within the rates tested in this experiment.

Taproot length also showed a quadratic trend. It appears that there was no improvement in taproot length up to a rate of 4 g/pot, and a decrease of the taproot length as amendment rate increased after 4 g/pot (r^2= 0.35, $P \leq 0.05$) (Table 6.5).

Root fresh or dry weight tended to respond positively (Table 6.5) to amendment rate until a certain level of amendment. This suggests that the root system might better tolerate the infection of the nematode if the level of the nematode remained the same under different amendment rates. This hypothesis might be useful to understand why some results show that use of organic amendments promoted plant growth as well as increased plant yield, but did not necessarily reduce nematode populations (Ichinohe, 1985). On black pepper under amendment treatment, Ichinohe (1985) reported that plants had an increased root system as well as improved vigor. Miller and Edgington (1962)

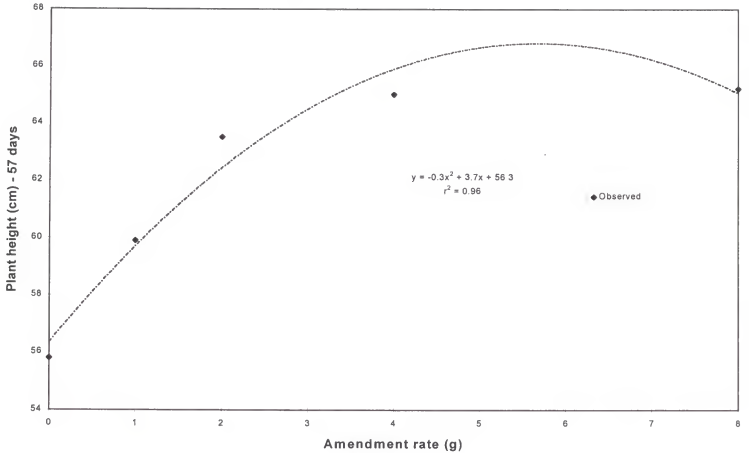

Figure 6.5. Quadratic relationships between okra height at 57 days and rate of velvetbean amendment, at 0 J2/pot and 1,000 J2/pot. Velvetbean experiment. Points = actual data; lines = fitted regression curve.

Table 6.5. Effect of velvetbean soil amendment rates (0, 1, 2, 4, or 8 g) on plant parameters of 'Clemson Spineless' okra. Summer 1996.

Amendment rate (g)	Plant height (cm) 57 days	Top weight (g) 35 days Fresh	35 days Dry	57 days Fresh
0	55.8†	9.05	1.36	9.79
1	59.9	11.82	1.75	11.33
2	63.5	11.56	1.71	13.84
4	65.0	13.25	1.70	15.30
8	65.2	16.19	1.86	20.61
Regression	$Y = -0.3X^2 + 3.7X + 56.3$	$Y = -0.04X^2 + 1.14X + 9.7$	$Y = -0.008X^2 + 0.11X + 1.5$	$Y = 1.3X + 10.2$
	$r^2 = 0.96$	$r^2 = 0.94$	$r^2 = 0.62$	$r^2 = 0.98$
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.05$	$P \leq 0.01$

† Data are means of ten replications, pooled across inoculum levels.

Table 6.5. (Continued ....)

Amendment rate (g)	Taproot length (cm) 57 days	Root weight (g) 35 days Fresh	57 days Fresh	57 days Dry
0	27.4†	3.99	5.99	0.55
1	34.9	4.95	6.24	0.62
2	30.3	4.93	8.23	0.75
4	29.2	5.03	8.20	0.84
8	26.6	6.16	10.48	1.03
Regression	$Y = -0.15X^2 + 0.8X + 30.0$	$Y = -0.006X^2 + 0.3X + 4.3$	$Y = -0.02X^2 + 0.76X + 6.0$	$Y = -0.004X^2 + 0.09X + 0.5$
	$r^2 = 0.35$	$r^2 = 0.86$	$r^2 = 0.92$	$r^2 = 0.99$
Significance	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

† Data are means of ten replications, pooled across inoculum levels.

reported reduction of *Pratylenchus penetrans* by using soil amendments, but also reported a better root system under their amendment treatments.

Inoculum level affected plant height ($P \leq 0.01$), taproot length ($P \leq 0.05$), and root weight ($P \leq 0.01$) (Table 6.3). The highest responses of plant height and taproot length were registered at 0 J2/pot, but greater root weight was recorded at 1,000 J2/pot (Table 6.6). These results are in accordance with the findings in previous experiments described in Chapters 2, 3, 4, and 5.

Table 6.6. Effect of inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* on 'Clemson Spineless' okra in a greenhouse experiment - Velvetbean experiment. Summer 1996.

Inoculum level	Plant height (cm)	Taproot length (cm)		Root weight (g)		
				Fresh		Dry
	57 days	35 days	57 days	35 days	57 days	57 days
0 J2/pot	64.12 a	29.7 a	32.0 a	4.05 b	5.73 b	0.67 b
1,000 J2/pot	59.64 b	26.8 b	27.4 b	5.98 a	9.93 a	0.84 a

† Data are means of five replications. Means followed by the same letters in columns are not significantly different ($P \geq 0.05$), according to Tukey's test.

Gall formation 35 days after inoculation ($P \leq 0.01$), J2 extracted from the egg masses ($P \leq 0.01$) at 57 days, and J2 extracted from the soil ($P \leq 0.05$) 57 days after inoculation responded negatively to amendment rate (Table 6.7). These parameters were best fit by quadratic regressions with $r^2 = 0.88$, 0.75, and 0.87, respectively (Table 6.7).

Egg mass ratings at 35 days (mean= 4.08) and 57 days (mean= 4.01), gall ratings at 57 days (mean= 4.96), and J2 extracted from egg masses at 35 days (means= 47.8) were not affected by amendment rate. Gall rating at 35 days, J2 extracted from the egg

Table 6.7. Effect of velvetbean soil amendment rates on *Meloidogyne arenaria* on 'Clemson Spineless' okra in a greenhouse experiment. Summer 1996.

Amendment	Egg mass rating[§]		Gall rating		J2[†] from egg masses		J2/100 cm^3 soil
rate (g)	35 days	57 days	35 days	57 days[§]	35 days[§]	57 days	(57 days)
0	4.2	4.1	4.8[††]	5.0	61.4	1199	362.3
1	4.2	4.2	4.6	5.0	89.4	812	200.4
2	4.0	4.0	4.0	5.0	31.4	753	104.2
4	4.0	4.0	4.0	4.8	39.8	769	109.2
8	4.0	4.0	4.0	5.0	17.0	643	50.2
Regression			$Y = 0.03X^2 - 0.3X + 4.8$			$Y = 13.4X^2 - 160X + 1088$	$Y = 8.4X^2 - 100X + 323$
			$r^2 = 0.88$			$r^2 = 0.75$	$r^2 = 0.87$
Significance	P≥0.05	P≥0.05	P≤0.01	P≥0.05	P≥0.05	P≤0.01	P≤0.01

† J2= second-stage juveniles.

†† Data are means of five replications.

§ Data were not significant at P≤0.05.

masses at 57 days, as well as J2 extracted from the soil decreased as amendment rate increased, therefore there was a suppression of the nematode with use of the amendment (Table 6.7). Nonetheless, at higher rates of the amendment under these experimental conditions, there were no additional effects of the amendment to further reduce the nematode population. This observation may be due to the fact that there was much more root system available at higher rates of the amendments, which provided more sites for nematode infection and reproduction (Table 6.5).

In general, under these experimental conditions, plant parameters responded positively to the amendment rate until a level of about 4 to 5 g of velvetbean per pot. Above this rate, there was a tendency for a decreasing response, represented by the quadratic trend. This model suggests an optimum rate of the amendment which promoted the best plant response, and beyond that rate the amendment might be toxic to the plant. This trend was also similar for the nematode evaluation. There was a suppression of the nematode, but beyond that optimum amendment rate, small or no changes in nematode dynamics occurred. Normally, the best responses of plant growth and nematode suppression under the velvetbean amendment were recorded at the rates of 4 to 6 g of the amendment per pot.

Experiment 2 (Castor amendment)

There was a significant interaction ($P \leq 0.01$) between inoculum level and amendment rate for top dry weight, at 60 days after inoculation, and for root fresh or dry weight at 37 and 60 days after inoculation (Table 6.8). The relationships between these

Table 6.8. Effect of inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* and castor soil amendment rates (0, 1, 2, 4, or 8 g) on plant parameters of 'Clemson Spineless' okra in a agreenhouse experiment. Data shown are F-values from the analysis of variance. Summer 1996.

Treatment	Stem diameter		Plant height		Top weight				Taproot lenght		Root weight			
	37	60	37	60	37 days		60 days		37	60	37 days		60 days	
effect	days	days	days	days	Fresh	Dry	Fresh	Dry	days	days	Fresh	Dry	Fresh	Dry
Inoculum (A)	11.2**	24.7**	2.9 ns	100.6**	17.8**	3.5 ns	85.3**	306.3**	1.2 ns	3.0 ns	7.4**	170.3**	324.6**	8.6**
Rate (B)	74.7**	129.3**	10.6**	46.7**	149.3**	56.7**	143.4**	96.5**	0.4 ns	1.0 ns	45.7**	58.1**	209.6**	115.7**
Interaction (AB)	0.7 ns	1.3 ns	0.8 ns	1.3 ns	0.7 ns	0.8 ns	2.1 ns	6.1**	1.4 ns	1.2 ns	4.0**	5.7**	24.2**	9.0**

*, ** Indicate significant main effect or interaction at P≤0.05 and P≤0.01, respectively, ns= effect not significant at P≥0.05.

plant parameters and amendment rate were better fit by quadratic regressions (Table 6.9), which had higher r^2 values than corresponding linear models. Biologically, a quadratic regression supports the hypothesis that at higher rates of the organic amendment the breakdown of the amendment might cause adverse effects, such as toxicity, to the plant (Table 6.9). Much of the research that investigated the use of organic amendments for biological control is dependent upon the amendment rate as well as the C/N ratio. Other workers have concluded that the breakdown of the amendment might be toxic to the plant first, even before suppression of the nematode (Trivedi and Barker, 1982; Muller and Gooch, 1982; Brown, 1987; Rodríguez-Kábana, 1986; Stirling, 1991).

Evidence that the organic amendment improved plant growth up to a point was very clear from the interaction responses. For instance, the top dry weight at 60 days after inoculation in the 1,000 J2/pot treatment showed only a relatively small change when amendment rate was increased from 4 to 8 g, similar to that which occurred under no inoculum at the same rates of the amendment (Table 6.9). Root fresh weight at 37 or 60 days after inoculation increased with amendment rate until a certain level of the amendment. However, in non-inoculated plants, there was not much additional effect on root weight when amendment rate was increased from 4 to 8 g/pot (Table 6.9). Thus, our results suggest that a higher volume of the root system expressed by the higher root weights may be available for nematode infection, but while there may still be root system to be infected, other factors might have interacted to prevent nematode infection (like toxicity of the amendment to nematodes, when it was in the process of breaking down). This hypothesis is supported by the findings of Singh and Sitaramaiah (1991), that soil

Table 6.9. Effect of castor soil amendment rates (0, 1, 2, 4, or 8 g) and inoculum density of second-stage juveniles (J2) of *Meloidogyne arenaria* on 'Clemson Spineless' okra. Summer 1996.

Amendment rate (g)	Top dry weight (g) - 60 days		Root weight (g) - 37 days			
			Fresh		Dry	
	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot
0	2.1†	0.6	2.7	4.6	0.26	0.62
1	3.2	1.5	5.8	6.0	0.48	0.64
2	3.8	1.7	5.6	6.8	0.61	1.19
4	5.0	2.8	7.9	7.0	0.72	1.05
8	6.3	3.1	8.0	8.7	0.78	1.15
Regression	$Y = -0.05X^2 + 0.9X + 2.2$	$Y = -0.05X^2 + 0.7X + 0.6$	$Y = -0.15X^2 + 1.8X + 3.2$	$Y = -0.04X^2 + 0.8X + 4.9$	$Y = -0.01X^2 + 0.2X + 0.3$	$Y = -0.02X^2 + 0.2X + 0.6$
	$r^2 = 0.99$	$r^2 = 0.98$	$r^2 = 0.92$	$r^2 = 0.93$	$r^2 = 0.97$	$r^2 = 0.70$
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

† Data are means of five replications.

Table 6.9. (Continued ...)

Amendment rate (g)	Root weight (g) - 60 days			
	Fresh		Dry	
	0 J2/pot	1,000 J2/pot	0 J2/pot	1,000 J2/pot
0	4.2[†]	5.0	0.50	0.43
1	5.8	9.8	0.77	0.82
2	7.4	11.9	0.96	0.97
4	10.3	15.9	1.26	1.28
8	10.8	19.5	1.21	1.68
Regression	$Y = -0.2X^2 + 2.2X + 4.0$	$Y = -0.24X^2 + 3.6X + 5.6$	$Y = -0.02X^2 + 0.3X + 0.5$	$Y = -0.01X^2 + 0.3X + 0.5$
	$r^2 = 0.99$	$r^2 = 0.99$	$r^2 = 0.99$	$r^2 = 0.99$
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

† Data are means of five replications.

amendments release a wide variety of chemically different substances, some of which may be toxic to nematodes. In addition, these authors mentioned that products released through amendment decomposition may influence the physiology of the plant, consequently affecting plant growth. Rapid and high root growth can mask the effect of the nematode injury. These results were in agreement with the findings in the first experiment (velvetbean amendment). In addition, Ichinohe (1985) stressed that mulching increases the amount of roots and plant vigor, so that plant yield responses can be higher even though the nematode level has not decreased. In most circumstances, quadratic equations best expressed the relationships between plant parameters and rates of velvetbean or castor amendment, which leads to the assumption that a best rate of the amendment for plant growth or nematode suppression can be predicted. Above the optimum amendment rate, there will be toxicity to the plant or no reduction of the final nematode population.

There was a significant ($P \leq 0.01$) main effect of the amendment rate on the following plant parameters: stem diameter, plant height, and top weight at 37 days after inoculation (Table 6.8). The relationships between those plant parameters and amendment rates were best described by quadratic regressions ($P \leq 0.01$) (Table 6.10). These results are in accordance with the findings in the first experiment. In addition, these relationships might suggest an adverse effect on plant growth above certain rates. The breakdown of the amendment may be toxic to the plant, since the control and inoculated treatments showed the same trends. Time of exposure to the amendment was another factor to be considered. Plants harvested 60 days after inoculation had higher

Table 6.10. Effect of castor amendment rates (0, 1, 2, 4, or 8 g) on stem diameter, plant height, and top weight of 'Clemson Spineless' okra in a greenhouse experiment. Summer 1996.

Amendment	Stem diameter (mm)		Plant height (cm)	
rate (g)	37 days	60 days	37 days	60 days
0	3.82†	3.92	36.9	47.9
1	4.25	4.64	41.7	55.9
2	4.55	4.95	44.4	57.0
4	5.05	5.67	43.7	64.9
8	5.67	6.36	44.4	67.5
Regression	$Y = -0.02X^2 + 0.4X + 3.8$	$Y = -0.03X^2 + 0.6X + 4.0$	$Y = -0.03X^2 + 2.8X + 38.1$	$Y = -0.4X^2 + 5.7X + 48.7$
	$r^2 = 0.99$	$r^2 = 0.99$	$r^2 = 0.80$	$r^2 = 0.97$
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

† Data are means of ten replications, pooled across inoculum levels.

Table 6.10. (Continued...)

Amendment rate (g)	Top weight (g)		
	37 days		60 days
	Fresh	Dry	Fresh
0	6.29†	1.04	7.23
1	10.36	1.71	11.61
2	13.56	2.12	13.96
4	16.15	2.35	20.91
8	19.85	2.65	28.63
Regression	$Y = -0.23X^2 + 3.4X + 6.8$	$Y = -0.04X^2 + 0.5X + 1.1$	$Y = -0.16X^2 + 4.0X + 7.3$
	$r^2 = 0.98$	$r^2 = 0.96$	$r^2 = 0.99$
Significance	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$

† Data are means of ten replications, pooled across inoculum levels.

values for those plant parameters than plants harvested 37 days after inoculation, and in some cases, such as for top dry weight (Tables 6.10, 6.11), showed more differences between inoculated and control treatments. These observations probably resulted because the amendment had much more time to be decomposed after 60 days, than when it was exposed for only 37 days. These results are in accordance with findings of Miller and Edgington (1962).

Table 6.11. Effect of inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* on 'Clemson Spineless' okra in greenhouse. Castor experiment. Summer 1996.

Inoculum	Stem diameter (mm)		Plant height (cm)	Top fresh weight (g)	
level	37 days	60 days	60 days	37 days	60 days
0 J2/pot	4.79 a†	5.3 a	63.8 a	12.44 b	19.4 a
1,000 J2/pot	4.54 b	4.9 b	53.5 b	14.05 a	13.6 b

† Data are means of five replications. Means followed by the same letters in columns are not significantly different at (P≤0.05), according to Tukey's test.

Inoculum level affected stem diameter, plant height, and top fresh weight (P≤0.01) (Table 6.8). The highest values were recorded at 0 J2/pot for these plant parameters, except for top fresh weight at 37 days after inoculation (Table 6.11).

Egg mass index at 37 days, J2 extracted from the egg masses at 37 days, and J2 extracted from soil at 60 days were affected by amendment rate (P≤0.01) (Table 6.12). These relationships were best described by quadratic regressions (Table 6.12). It can be shown that at rates between 4 to 6 g of castor per pot, the relationship predicts maximum suppression of egg mass formation or J2 extracted from soil. However, for J2 extracted from the egg masses, suppression was still progressing up to 8g of the amendment per

Table 6.12. Effect of castor soil amendment rates on *Meloidogyne arenaria* on 'Clemson Spineless' okra in a greenhouse experiment. Summer 1996.

Amendment	Egg masses rating		Gall rating[§]		J2[†] from egg masses		J2/100 cm^3 soil
rate (g)	37 days	60 days[§]	37 days	60 days[§]	37 days[§]	60 days[§]	60 days
0	4.6[††]	4.0	4.6	5.0	653	1,230	362.32
1	4.8	4.0	4.8	5.0	820	997	200.4
2	4.2	4.0	4.2	5.0	729	1,210	104.2
4	4.0	4.0	4.0	5.0	562	748	109.2
8	4.0	4.2	4.0	5.0	424	724	50.2
Regression	$Y= 0.02X^2 - 0.3X + 5$				$Y= - 3.8X^2 - 10X + 732$		$Y= 3.3X^2 - 39X + 131$
	$r^2= 0.77$				$r^2= 0.76$		$r^2= 0.93$
Significance	P≤0.01	P≥0.05	P≥0.05	P≥0.05	P≤0.01	P≥0.05	P≤0.01

† J2= second-stage juveniles.

†† Data are means of five replications.

§ Data were not significant at P≤0.05.

pot. Egg mass index at 60 days (mean= 4.72), gall ratings at 37 days (mean= 4.04), and 60 days (mean= 5.0), and J2 from the egg masses at 60 days (mean= 971.75) were not affected by amendment rate.

In general, under these experimental conditions, plant parameters increased progressively in response to the amendment rate until a certain level, beyond which there was a tendency of a decreasing response. This trend was represented by the quadratic regression, for which the coefficients were fitted with high r^2. The decreasing response suggests that the amendment might be toxic to the plant because even in the control treatments without nematodes, the trend was similar. Normally, the best responses of plant growth and of nematode suppression under the castor amendment were recorded at rates of 4 to 6 g of the amendment per pot.

The results from the first and the second experiments lead to the conclusion that the longer the amendment was in the soil, the more effective it was. For both experiments, more effects on plant parameters were observed during the second harvest of the experiment than during the first harvest. The castor amendment had a C/N ratio slightly lower than the velvetbean amendment (Table 6.1), which might explain why responses might have differed for some of the plant parameters or nematode evaluations. However, it should be considered that castor amendment (6.9 g fresh: 1 g dry) was less dense than velvetbean (4.4 g fresh: 1 g dry).

Experiment 3 (Combined amendments, velvetbean and castor)

In comparision with unamended controls, velvetbean or castor amendments resulted in greater values for plant parameters and the smaller for the nematode numbers. For instance, plants without amendment (average of six replications) had plant heights of only 4.90 cm at 0 J2/pot and 4.01 cm at 1,000 J2/pot, compared with heights of 9.38 cm at 0 J2/pot and 9.22 cm at 1,000 J2/pot under amendment (Data not shown). For the nematode evaluations, egg mass index was 5.0 in unamended vs. 4.6 in amended treatments, root gall index was 4.83 in unamended vs. 4.7 in amended, juveniles extracted from 10 egg masses averaged 1,330 from unamended vs. 1,283 from amended, and juveniles per 100 cm^3 soil averaged 86.2 from unamended vs. 11.4 from amended treatments.

There was a triple interaction ($P \leq 0.05$) among inoculum level, amendment type, and amendment rate for stem diameter at 45 days after inoculation (Table 6.13). Stem diameter was greater with 8 g of velvetbean amendment than with 2 g, but rates of castor did not affect stem diameter (Table 6.14).

There was a significant interaction ($P \leq 0.01$) between amendment and amendment rate for all plant parameters except for taproot length (Table 6.13). There was no significant difference between stem diameter (30 or 45 days after inoculation) under the castor amendment at rates of 2 or 8 g/pot, but stem diameter was higher at rate of 8 g/pot of velvetbean amendment. Plant height at 30 days after inoculation was higher with velvetbean amendment, and the highest value was registered under 8 g/pot. The same

Table 6.13. Effect of organic amendment, amendment rate, and inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* on 'Clemson Spineless' okra in a greenhouse experiment. Data shown are F-values from the analysis of variance. Combined experiment.

Treatment effect	Stem diameter		Plant height		Top weight		Taproot	Root weight	
	30 days	45 days	30 days	45 days	Fresh	Dry	length	Fresh	Dry
Amendment (A)	2.15 ns	2.57 ns	5.94*	5.95*	9.65**	5.36*	1.30 ns	3.53 ns	2.90 ns
Amendment rate (B)	8.99**	15.13**	1.11 ns	11.34**	40.43**	3.24 ns	0.01 ns	1.50 ns	0.00 ns
Inoculum level (C)	8.24**	0.05 ns	4.83*	1.84 ns	0.12 ns	9.58**	27.79**	0.04 ns	0.58 ns
Interaction (AB)	16.15**	25.34**	6.24*	12.96**	28.50**	15.23**	3.74 ns	12.70**	16.23**
Interaction (AC)	0.20 ns	2.57 ns	0.15 ns	0.66 ns	0.05 ns	0.95	0.00 ns	2.08 ns	0.53 ns
Interaction (BC)	2.54 ns	0.47 ns	0.15 ns	0.04 ns	0.10 ns	0.46	0.02 ns	2.93 ns	2.66 ns
Interaction (ABC)	0.04 ns	5.24*	0.04 ns	0.00 ns	0.32 ns	2.00 ns	0.31 ns	0.65 ns	3.78 ns

*, ** Indicate significant main effect or interaction at $P \leq 0.05$ and $P \leq 0.01$, respectively, ns= effect not significant at $P \geq 0.05$.

trend occurred with plants measured 45 days after inoculation. Top fresh and dry weight, and root fresh and dry weight were also highest with the velvetbean amendment at rate of 8 g/pot (Table 6.15).

There was a significant interaction ($P \leq 0.05$) between amendment and amendment rate for galls per g dry root (Table 6.16). The highest number was recorded at 8 g of the

Table 6.14. Effect of the amendment, amendment rate, and inoculum level of second-stage juveniles (J2) of *Meloidogyne arenaria* on stem diameter (cm) at 45 days and top fresh weight (g) of okra. Combined experiments.

Amendment rate	Stem diameter (cm)		
	Velvetbean	Castor	Mean
	---------- *0 J2/pot* ----------		
2	3.37 b†	3.67 a	3.52
8	4.13 a	3.37 a	3.75
Mean	3.75	3.52	3.63
	---------- *1,000 J2/pot* ----------		
2	3.38 b	3.58 a	3.48
8	3.92 a	3.72 a	3.82
Mean	3.65	3.65	3.65

† Data are means of six replications. Means followed by small letters in columns are not significantly different at $P \leq 0.05$, according to Tukey's test. Capital letters refer to comparisons between main-effect means in columns or in rows.

castor amendment. Levels recorded at 2 or 8 g of velvetbean amendment were similar to that for 2 g of castor amendment (Table 6.17). Egg mass index was affected by the amendment rate ($P \leq 0.05$). Egg mass formation was suppressed at 8 g of the amendment per pot (Table 6.17). Juveniles (J2) extracted from soil were affected ($P \leq 0.05$) by the

Table 6.15. Effect of amendment and amendment rates (2 or 8 g) on 'Clemson Spineless' okra in a greenhouse experiment. Combined experiment.

Amendment rate	Velvetbean	Castor	Mean	Velvetbean	Castor	Mean	Velvetbean	Castor	Mean	Velvetbean	Castor	Mean
	---Stem diameter (mm) 30-days --			*--- Stem diameter (mm) 45-days ---*			*---- Plant height (cm) 30-days ----*			*----- Plant height (cm) 45-days ----*		
2	3.3 b†	3.5 a	3.4 B	3.4 b	3.6 a	3.5 B	33.6 b	33.7 a	33.6 A	37.4 b	38.7 a	38.0 B
8	3.8 a	3.4 a	3.6 A	4.0 a	3.5 a	3.8 A	36.1 a	32.7 a	34.4 A	44.9 a	38.4 a	41.7 A
Mean	3.5 A	3.4 A	3.5	3.7 A	3.6 A	3.6	34.8 A	33.2 B	34.0	41.2 A	38.5 B	39.8
	------- Top fresh weight (g) --------			*--------- Top dry weight (g) ---------*			*------- Root fresh weight (g) -------*			*--------- Root dry weight (g) --------*		
2	7.4 b	8.4 a	7.9 B	1.29 b	1.44 a	1.4 A	3.4 b	3.8 a	3.6 A	0.3 b	0.4 a	0.3 A
8	12.6 a	8.8 a	10.7 A	1.80 a	1.25 a	1.5 A	4.4 a	3.3 a	3.8 A	0.4 a	0.3 b	0.3 A
Mean	10.0 A	8.6 B	9.3	1.55 A	1.34 B	1.4	3.9A	3.5 A	3.7	0.4 A	0.3 A	0.3

† Data are means of six replications. Means followed by small letters in columns are not significantly different at P≤0.05, according to Tukey's test. Capital letters refer to comparisons between main effect means in columns or in rows.

amendment type (Table 6.16). Velvetbean amendment was more efficient in suppressing J2 from soil than castor amendment (Table 6.17). Juveniles hatched from 10 egg masses and eggs per g dry root did not differ between castor and velvetbean amendments (Table 6.17).

In general, responses of plant parameters or nematode evaluations varied with amendment and amendment rate. Plant measurements were generally highest under 8 g of velvetbean amendment. No significant ($P \geq 0.05$) differences were recorded between

Table 6.16. Effect of organic amendments and amendment rates (0, 2, or 8 g) on *Meloidogyne arenaria* infecting 'Clemson Spineless' okra in a greenhouse experiment. Data shown are F-values from the analysis of variance. Combined experiments.

Treatment effect	Juveniles (J2)		Egg masses		Galls	
	J2/10 egg masses	J2/100 cm^3 soil	per root system	per g dry root	per root system	per g dry root
Amendment (A)	2.52 ns	7.01*	0.83	1.71 ns	3.64 ns	4.57*
Amendment rate (B)	1.15 ns	0.53 ns	7.50*	0.10 ns	0.91 ns	4.06 ns
Interaction (AB)	1.04 ns	0.62 ns	0.83 ns	4.22 ns	0.00 ns	4.35*

*, ** Significant effects at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns= not significant.

rates of 2 or 8 g of castor amendment, which resulted in levels of plant and nematode parameters similar to those obtained with 2 g of velvetbean amendment.

In the first and second experiments, there were significant responses of plant parameters and nematodes to velvetbean and castor amendments applied at low rates. However, at the highest rate (8 g/pot), there were small differences or no additional effects on plant parameters or nematode suppression. Thus, the differences recorded

Table 6.17. Effect of amendment type and amendment rate (2 or 8 g) on *Meloidogyne arenaria*. Combined experiments. September 1996.

Amendment rate	Velvetbean	Castor	Mean	Velvetbean	Castor	Mean	Velvetbean	Castor	Mean
	Egg mass index			*Gall index*			*J2/10 egg mass*		
2	4.8† a	4.8 a	4.8 A	4.5 a	4.8 a	4.7 A	1433 a	1220 a	1326 A
8	4.2 a	4.5 a	4.3 B	4.7 a	5.0 a	4.8 A	1262 a	1216 a	1239 A
Mean	4.5 A	4.7 A	4.6	4.6 A	4.9 A	4.7	1348 A	1218 A	1283
	Eggs/g dry root			*Galls/g dry root*			*J2/100 cm3 soil*		
2	14.8 a	13.6 a	14.2 A	13.5 a	13.6 b	13.6 A	7.5 a	17.3 a	12.4 A
8	12.1 a	17.3 a	16.2 A	13.5 a	19.0 a	16.2 A	7.7 a	13.0 a	10.3 A
Mean	13.4 A	15.4 A	14.4	13.5 B	16.3 A	14.9	7.6 B	15.2 A	11.4

† Data are means of six replications. Means followed by small letters in columns are not significantly different at P≤0.05, according to Tukey's test. Capital letters refer to comparisons between means in columns or in rows.

between 2 or 8 g of velvetbean amendment in the third experiment are somewhat remarkable. This suggest that, under the conditions of the third experiment, in which both amendments were compared directly, velvetbean amendment may be more efficient in suppressing nematodes as well as in improving plant growth. Possibly in the process of the amendment breakdown, velvetbean may release substances that improve plant growth and suppress nematodes better than the castor amendment, since all the other parameters were constant. In addition, it was necessary to use only 4.4 g of fresh velvetbean amendment to obtain 1 g of dry velvetbean, compared to 6.9 g of fresh castor. Experimental results demonstrated that 2 g of velvetbean amendment per pot did not differ from 2 or 8 g of castor amendment per pot. The greater density of the velvetbean amendment compared to the castor amendment would suggest that less velvetbean amendment will be necessary to promote plant growth and nematode suppression.

CHAPTER 7
EFFECT OF NEMATODE LEVEL (*MELOIDOGYNE ARENARIA* RACE 1) AND MULCH TYPE ON 'CLEMSON SPINELESS' OKRA IN MICROPLOT EXPERIMENTS

Introduction

Since early reports of reductions of nematode populations by using organic amendments (Linford et al., 1938; Watson and Goff, 1937; Holtz and Vandecaveye, 1938; Watson, 1945), the interest in applying organic amendments to manage plant-parasitic nematodes has increased considerably (Johnson, 1959; Lear, 1959; Mankau and Minteer, 1962; Singh et al., 1967; Muller and Gooch, 1982; Miller at al., 1968; Johnson and Shamiyeh, 1975; Rodriguez-Kabana, 1986; Stirling, 1991; McSorley and Gallaher, 1995b). Nonetheless, the use of amendments could be promoted more widely if the mechanism involved in nematode suppression and the amount of organic amendment necessary for suppression of the plant-parasitic nematode were better known, (McSorley and Gallaher, 1995b).

The plant-parasitic nematode population under organic amendment treatments may change for many different reasons, including changes in soil properties, nutrients released to plants, increase of predacious microorganisms, toxic metabolites released from organic amendment breakdown, or absence of a host crop. Nonetheless, changes are still dependent on the C/N ratio of the organic amendment, nematode species, and level of amendment needed for nematode suppression or plant tolerance. Thus, the objective of this study was to verify the effect of two organic amendments (with

different C/N ratios) and a control treatment (without amendment) and two population levels of *Meloidogyne arenaria* (Neal) Chitwood race 1 on a highly susceptible crop and on nematode populations. The experiment was conducted in microplots under field conditions over a period of three years, during which microplots were planted with a root-knot nematode susceptible vegetable crop in the summer and a cover crop (poor host) in the winter.

Materials and Methods

The experiments were carried out at the University of Florida, Green Acres Agronomy Farm, in Alachua County, Florida, during 1994, 1995, and 1996. Experiments were conducted in fiberglass microplots (Johnson et al., 1981), 75 cm in diameter and 50 cm deep and open at the bottom. The soil was characterized as 92% sand, 6% clay, and 2% silt.

On 26 May 1994, 24 microplots were cleaned and sampled for counting the initial nematode population. The nematodes were extracted and quantified from five soil cores per microplot by the centrifugal flotation technique (Jenkins, 1964). The microplots were then characterized as having a low or high level of *Meloidogyne arenaria*, based on the initial count of juveniles (J2) extracted from 100 cm^3 soil.

Initial population of *M. arenaria* in the 12 plots defined as low level averaged 1.58 (sd= 2.8), and in the 12 plots defined as high level, averaged 303 (sd= 187) *M. arenaria* per 100 cm^3 soil. Other plant-parasitic nematodes were also counted in case they could be also harmful to the experimental crop, regardless of the level of *M. arenaria* race 1.

On 13 June 1994, a composite soil sample from each nematode level (high, low) was taken, J2 were extracted, and a random sample of 100 J2 was examined to confirm that J2 were free of *Pasteuria.*

In each year, the nematode susceptible 'Clemson Spineless' okra (*Hibiscus esculentus* L.) was used for the summer season as a test plant, and 'Wrens Abruzzi' rye (*Secale cereale* L.) for the cover crop in the fall/winter season, because this crop is not a good host of root-knot nematode (McSorley, 1994). For each year, okra seeds were individually sown in Speedling ^{TM} trays (27 cm x 52 cm, with capacity for 36 seedlings) in a growth room, according to procedures described in Chapter 6. Each microplot received 7 two-week-old okra seedlings. After harvest, microplots were cleaned of okra residues, and two spoons of 'Wrens Abruzzi' rye were sown in each plot. Each spoon held about 275 seeds, weighing about 7.3 g. The germination rate was about 64%. Transplanting and harvesting dates for okra differed for each year, nonetheless data were collected by the same procedures to avoid variability. In the first year, on 20 June 1994, each microplot was fertilized by applying 40 g of 13-4-8 (N-P_2O_5-K_2O) fertilizer, before the treatments were established. In subsequent years, no fertilizer was applied. Irrigation and occasional insecticidal sprays to control grasshoppers were applied as needed.

The experimental design was a 2 x 3 factorial with two nematode levels and three mulch types, replicated four times. The organic amendment treatments consisted of an aged yard-waste compost (mainly woodchips) (McSorley and Gallaler, 1995a), perennial peanut (*Arachis glabrata* Benth.) hay, and a control treatment, without amendment. The amendment treatments were applied as mulches, and consisted of dry volumes of 9,000 cm^3 of perennial peanut hay or woodchips weighing 1,474 g and 850 g, respectively.

These amendments were applied only in the first year, right after okra seedlings were transplanted. The additional amendment in each plot was due to the hay from the rye cover crop, which was cut, weighed, and left on the plot. Samples of perennial peanut hay, woodchips, and rye hay were taken for mineral analysis according to the methodology previously described in Chapter 2 (Gallaher et al., 1975; Walkley and Black, 1934; Walkley, 1947, Mehlich, 1953), and for determination of C/N ratio (J. R. Rich, North Florida Research and Education Center, Quincy, FL., pers. comm.). Results are presented in Table 7.1.

In each year, okra fruit were harvested at a minimum size of 6 cm and they were picked at least two times per week, to avoid great variability among treatments. Plant height and stem diameter were measured twice after transplanting. Stem diameter was measured at a height of 10 cm, and plant height was measured from plant base to apical meristem. Data on yield consisted of average pod size, number of pods, and total weight of pods per microplot. Root galling and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0= 0 galls or egg masses per root system, 1= 1-2, 2= 3-10, 4= 11-30, 4= 31-100, 5= more than 100 galls or eggs masses per root system.

For each crop, five soil cores were taken per microplot to estimate the initial and final nematode population on okra each year. Nematodes were extracted from 100-cm^3 soil subsamples following the centrifugal flotation technique (Jenkins, 1964). Nematode count data were log-transformed ($\log_{10}[x + 1]$) before data analysis, but only untransformed arithmetic means are presented in tables.

Data were statistically analyzed as a 2 x 3 factorial to determine the main effect and interaction between mulch and nematode level. When the main effect and interaction

Table 7.1. Mineral analysis and C/N ratio for each organic amendment. Data are means of five replications.

Amendment	C/N ratio	Macronutrients					Micronutrients			
		N	K	Ca	Mg	P	Mn	Zn	Cu	Fe
		%			ppm					
Peanut hay	10.66	1.353	1.375	1.767	3,205	3,325	79	87.5	12	277
Woodchips	14.78	0.393	0.149	2.247	1,322	1,047	180	69.2	25	770
Rye hay	11.26	0.195	1.105	0.212	757	1,745	57	22.5	3	90

were significant, separate analyses of variance was carried out, followed by separation of the means by Tukey's test (SAS Institute, Cary, NC).

First Year - 1994

Okra seedlings were transplanted on 23 June. Immediately after transplanting, a layer of 2 cm of the appropriate organic amendment was distributed on each microplot, totaling 9,000 cm^3. Harvest of okra pods was initiated on 30 July and was finalized on 14 September, totaling 18 pickings. Data on stem diameter and plant height were recorded on 21 August and on 6 September, 58 and 75 days after transplanting, respectively.

On 15 September, the remaining plants in each microplot were removed carefully and brought to the laboratory for rating galls on the root system. Egg masses were not rated. On the same day, a soil sample was collected from each plot for extraction of nematodes from soil. Thus, microplots were cleaned of the okra residues, but any of the original organic amendment remaining was left in each microplot. On 16 October, another soil sample were taken for soil mineral analysis and after that, rye was sown. No additional irrigation or fertilizer was applied.

Second Year - 1995

On 17 May, rye hay was cut and the stems were chopped into pieces of about 20 cm, and left on each microplot until okra seedlings were transplanted. The rye seed heads were pulled from each microplot to avoid further germination. The rye straw was carefully taken from each microplot at the time of transplanting of okra, which occurred on 1 June. After transplanting, the rye straw was put back in each microplot. On 9 June,

each microplot received replanting of at least two seedlings because it was extremely dry, causing death of some seedlings.

On 10 June, the average depth of the rye layer was recorded based on 5 random measurements in the microplot. Stem diameter and plant height were recorded on 6 July and on 11 August, 36 and 72 days after okra transplanting. Harvesting began on 21 July and finished on 14 August, totaling 10 pickings. On 15 August, the experiment was terminated by the same procedure described in 1994, and soil samples were taken for evaluating the final nematode populations. Root systems were rated for egg masses and galls on the same day. However, okra tops were left in the microplots. On 4 November, plots were cleaned of the okra residues, and another soil sample were taken to constitute the initial nematode population for the cover crop, rye, by the same procedure described previously. An additional 100 cm^3 of soil was removed from each soil sampled, dried, and used for the mineral and organic matter analyses. Another 100 cm^3 of soil from each nematode treatment level was used to extract J2, from which 100 J2 were randomly sampled to verify that J2 were free of *Pasteuria*.

At the time of sowing of rye, all the organic amendment left in each microplot was removed for sowing of rye seed. After rye was sown, the remaining straw and amendment were placed back in each microplot. Immediately, another estimation of rye layer depth was done for each microplot following the same procedure described before. For this season, no fertilizer or supplemental irrigation were added.

Third Year - 1996

On 8 May, rye hay was cut, chopped, and left on each microplot, according to the same procedure described in 1995. However, rye heads and stems were weighed individually for each microplot. Soil for estimating the final nematode population was sampled on the same day. Thus, J2 extracted from the 100 cm^3 soil were computed as the final nematode population for the cover crop and at the same time served as a preplant nematode population for okra. The remaining soil sampled was dried and used for mineral analysis and organic matter determination.

Okra seedlings were transplanted on 30 May, and depth of the rye layer was estimated according to the same procedures described before. Stem diameter and plant height were recorded on 2 July and on 7 August, 33 and 69 days after okra transplanting. On 25 June, 26 days after transplanting, okra leaf blades and petioles were sampled for mineral analyses. Harvesting began on 11 July and was terminated on 7 August, totaling 10 pickings. The experiment was terminated on 7 August, and soil samples were taken for evaluating the final nematode populations.

Results

Okra Plant Growth

In the first year of the experiment, there were interactions between nematode level and amendment type for plant height at harvest ($P \leq 0.05$), total fruit weight ($P \leq 0.05$), and final stand ($P \leq 0.01$) (Table 7.2). For these variables, the highest values were recorded under the low nematode level and mostly with the peanut hay treatment (Table 7.3).

Table 7.2. Effect of nematode *(Meloidogyne arenaria)* population level and organic amendment on plant parameters of 'Clemson Spineless' okra in microplot experiments. Data shown are F-values from the analysis of variance.

Treatment effect	Stem diameter		Plant height		Fruit (total)			Final
	Midseason†	Harvest††	Midseason	Harvest	Number	Weight (g)	Size (cm)	Stand
					1994			
Nematode level (A)	33.24**	20.05**	105.10**	118.77**	108.82**	76.13**	5.65*	68.26**
Amendment (B)	83.03**	54.15**	74.75**	60.14**	17.19**	19.63**	1.93 ns	0.30 ns
Interaction (AB)	0.24 ns	0.26 ns	0.23 ns	4.62*	1.37 ns	5.08*	0.67 ns	7.38**
					1995			
Nematode level (A)	0.50 ns	2.87 ns	1.71 ns	12.93**	2.81 ns	5.03*	1.34 ns	0.31 ns
Amendment (B)	15.86**	18.00**	10.20**	9.06**	1.23 ns	1.27 ns	2.69 ns	1.37 ns
Interaction (AB)	0.68 ns	3.09*	0.75 ns	1.93 ns	0.03 ns	0 ns	0.44 ns	0.34 ns
					1996			
Nematode level (A)	0.89 ns	1.08 ns	0.76 ns	0.15 ns	0.01 ns	0.11 ns	0.79 ns	0.30 ns
Amendment (B)	8.16**	4.60*	2.37 ns	0.36 ns	1.07 ns	0.66 ns	1.94 ns	0.04 ns
Interaction (AB)	9.27**	10.02**	4.45*	15.69**	2.77 ns	2.61 ns	1.53 ns	3.38 @

*, ** Indicate significant main effect or interaction at P≤0.05 and P≤0.01, respectively, @ indicates significance at $0.05 \leq P \leq 0.10$, ns= effect not significant at P≤0.10.

† Midseason measurement taken at 58 days after transplanting in 1994, 36 days in 1995, and 33 days in 1996.

†† Harvest measurement taken at 75 days after transplanting in 1994, 72 days in 1995, and 69 days in 1996.

Table 7.3. Effect of nematode *(Meloidogyne arenaria)* population level and organic amendment on plant parameters of 'Clemson Spineless' okra in microplot experiments.

Amendment	Stem diameter (mm)						Plant height (cm)					
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
	-------- *01 June 1994* --------			------ *15 August 1994* ------			-------- *01 June 1994* ---------			-------- *15 August 1994* ------		
Control	7.00	4.36	5.84 b	7.33	4.52	6.77 b	34.0	20.0	27.0 b	40.9 Ab	20.7 Bb	30.8
Peanut hay	14.54	11.39	13.13 a	14.67	11.46	13.23 a	56.9	40.5	48.7 a	75.8 Aa	42.3 Ba	59.1
Woodchips	8.16	5.83	7.11 b	8.35	6.12	7.50 b	39.2	24.8	32.0 b	47.3 Ab	27.7 Bb	37.5
Mean	9.48A	6.83 B	8.30	9.74 A	8.11 B	9.17	43.3 A	28.4 B	35.6	54.7	30.3	45.9
	-------- *06 July 1995* ---------			------ *11 August 1995* -------			--------- *06 July 1995* ---------			-------- *11 August 1995* ------		
Control	4.01	3.69	3.86 b	6.19	5.64	5.92 b	18.9	18.1	18.5 a	32.7	27.9	30.3 b
Peanut hay	4.53	4.67	4.60 a	7.53	8.00	7.75 a	19.7	18.8	19.3 a	34.7	33.7	34.2 a
Woodchips	3.54	3.37	3.45 b	6.29	4.75	5.48 b	17.0	17.2	17.1 b	24.9	24.9	28.2 b
Mean	4.04	3.89	3.96	6.67	6.67	6.38	18.5	18.0	18.3	33.0 A	28.8 B	30.9
	------- *02 July 1996* -------			------ *07 August 1996* -------			--------- *02 July 1996*---------			------- *07 August 1996*-------		
Control	5.78 Aa	5.12 Ab	5.54	6.71 Aa‡	5.77 Bb‡	6.33	21.0 Aa‡	20.0 Ac‡	20.5	36.1 Aa	27.5 Bb	31.8
Peanut hay	5.32 Bab	6.22 Aa	5.79	6.17 Ba‡	7.45 Aa‡	6.95	20.3 Ba‡	22.5 Aa‡	21.4	29.0 Bb	36.8 Aa	32.9
Woodchips	4.96 Ab	5.12 Ab	5.04	5.94 Aa‡	6.24 Ab‡	6.11	20.2 Aa‡	20.3 Abc‡	20.2	32.1 Aab	31.5 Aab	31.8
Mean	5.35	5.49	5.42	6.33	6.60	6.47	20.5	20.9	20.7	32.4	31.9	32.8

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at 0.05≤P≤0.10.

Table 7.3. (Continued ....)

Amendment	Total number of fruit			Fruit length (cm)			Total fruit weight (g)			Final stand		
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
							1994 harvest§					
Control	22.2	1.5	11.9 b	8.5	6.5	7.5	284 Ab	11 Ba	147	7.0 Aa	1.2 Bb	4.1
Peanut hay	38.7	11.5	25.1 a	8.8	8.2	8.5	642 Aa	139 Ba	391	5.2 Aa	3.7 Aa	4.5
Woodchips	21.2	2.2	11.7 b	8.1	5.5	6.8	239 Ab	15 Ba	127	6.5 Aa	2.5 Bab	4.5
Mean	27.4 A	5.1 B	16.2	8.4 A	6.7 B	7.6	388	55	222	6.2	2.5	4.4
							1995 harvest					
Control	13.2	7.7	10.5	7.2	6.5	6.9	165	76	120	6.0	4.7	5.4
Peanut hay	13.2	9.2	11.2	21.7	11.3	16.5	187	102	145	4.2	4.5	4.4
Woodchips	8.7	3	5.9	7.1	3.1	5.1	115	22	70	4.0	3.7	3.9
Mean	11.7	7.0	9.2	12.0	7.0	9.5	156 A	68 B	112 A	4.7	4.3	4.5
							1996 harvest					
Control	12.0	4.2	8.1	9.4	8.4	8.9	159	39	99	6.7 Aa‡	4.5 Bb‡	5.6
Peanut hay	9.2	13.7	11.5	8.7	9.3	9.0	101	157	129	4.5 Bb‡	7.0 Aa‡	5.7
Woodchips	5.5	9.5	7.5	6.0	8.5	7.3	67	97	82	5.0 Bab‡	6.0 Aa‡	5.5
Mean	8.9	9.2	9.0	8.0	8.8	8.4	109	98	103	5.4	5.8	5.6

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different ($P \leq 0.05$), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at $0.05 \leq P \leq 0.10$.

§ Total of 18 pickings in 1994, and 10 pickings each in 1995 and 1996.

In the first season (1994), inoculum level affected all plant parameters ($P \leq 0.05$) (Table 7.2). Measurements in plots with the low nematode level were always greater than the corresponding values at the high nematode level (Table 7.3). However, differences with nematode level did not persist into the second and third seasons (Tables 7.2, 7.3).

Amendment Effect

The organic amendment used affected ($P \leq 0.05$) stem diameter at midseason and harvest in every season, and affected ($P \leq 0.01$) plant height on both sampling dates in the first two seasons (Table 7.2). The highest values were generally recorded under the peanut hay amendment (Table 7.3). Okra yields (number and weight of fruit) were also greatest in peanut-amended plots, but only in the first season (Table 7.3). In the third season, significant ($P \leq 0.05$) interactions between nematode level and organic amendment resulted from a greater response to peanut amendment in the plots with the high level of *M. arenaria* (Tables 7.2, 7.3).

Since no fertilizer was applied except in the first year, the organic amendments were of particular importance as potential sources of nutrients. Peanut hay was the richest source of N (Table 7.1), and probably provided a fertility source responsible for the improved plant growth.

Data recorded show that both stem diameter and plant height had a tendency to decrease each year. The average plant height at harvest varied from 45.9 cm in 1994 to 32.8 cm in 1996, and stem diameter varied from 9.2 mm to 6.4 mm. These declines in plant growth over time probably resulted as N from the original fertilizer and from peanut

hay were used up over time, since neither of these materials were reapplied. The total number of fruit or total fruit weight reduced drastically from the first year to the second, but seemed to stabilize in the third year.

Nematodes

In the first year, gall index and initial and final root-knot nematode populations were affected ($P \leq 0.01$) by nematode level (Table 7.4). This would be expected since the original treatment separated plots into high and low levels of root-knot nematodes. However, amendment treatment had no effect on nematode population or gall index ($P \geq 0.05$).

Differences in numbers of root-knot nematodes and in galling and egg mass indices between plots with high vs. low nematode levels were generally maintained in the second and third years of the experiment (Tables 7.4, 7.5, 7.6). However, in these years, amendment treatment affected ($P \leq 0.01$) gall and egg mass indices, and significant nematode level and amendment interactions were evident for these parameters as well (Table 7.4). These interactions resulted because galling and egg mass indices were lower with the control treatment at the low nematode level, but were not affected by organic amendment treatment at the high nematode level (Table 7.6).

The stubby-root nematode (*Paratrichodorus minor*) colonized these microplots (McSorley et al., 1994). In the first year of the study, stubby-root nematodes built up more quickly in plots with low levels of *M. arenaria* than in plots with high levels of *M.*

Table 7.4. Effect of nematode *(Meloidogyne arenaria)* population level and organic amendment on gall and egg mass indices of *Meloidogyne arenaria* race 1 on 'Clemson Spineless' okra and on numbers of root-knot (*M. arenaria*) and stubby-root (*Paratrichodorus minor*) nematodes in soil in microplot experiments. Data shown are F-values from the analysis of variance.

Treatment effect	Gall index	Egg mass index	Nematodes per 100 cm^3 soil			
			Root-knot		Stubby-root	
			Pi†	Pf‡	Pi	Pf
			1994			
Nematode level (A)	92.24**	.	289.16**	18.95**	8.50**	5.88**
Amendment (B)	1.74 ns	.	1.35 ns	1.32 ns	0.72 ns	0.08 ns
Interaction (AB)	0.39 ns	.	0.30 ns	0.54 ns	0.61 ns	1.19 ns
			1995			
Nematode level (A)	81.13**	49.82**	11.36**	13.52**	0.58 ns	0.75 ns
Amendment (B)	10.31**	3.66**	4.66*	2.45 ns	0.27 ns	0.84 ns
Interaction (AB)	11.03**	11.76**	0.30 ns	2.85 @	0.82 ns	0.37 ns
			1996			
Nematode level (A)	14.92**	3.93*	7.78 *	0.34 ns	0.92 ns	0.01 ns
Amendment (B)	4.75**	8.47**	0.16 ns	2.08 ns	1.92 ns	0.03 ns
Interaction (AB)	5.32**	3.39*	1.52 ns	3.43 @	1.23 ns	1.43 ns

*, ** Indicate significant main effect or interaction at $P \leq 0.05$ and $P \leq 0.01$, respectively, @ indicates significance at $0.05 \leq P \leq 0.10$, ns= effect not significant at $P \leq 0.10$.

† Initial nematode population (Pi) taken on 23 May 1994, 1 June 1995, and 8 May 1996.

‡ Final nematode population (Pf) taken on 17 September 1994, 15 August 1995, and 7 August 1996.

Table 7.5. Effect of the initial nematode (*Meloidogyne arenaria*) population level and organic amendment on numbers of root-knot (*Meloidogyne arenaria*) and stubby-root (*Paratrichodorus minor*) nematodes per 100 cm^3 soil in microplot experiments with 'Clemson Spineless' okra.

Amendment	Root-knot nematodes/100 cm^3 soil						Stubby-root nematodes/ 100 cm^3 soil					
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
	23 May 1994			*23 September 1994*			*23 May 1994*			*23 September 1994*		
Control	1.0	284	143	14	127	71	12	0	6	19	3.7	11
Peanut hay	0.2	242	121	66	169	118	6	0	3	10	5.0	7
Woodchips	3.5	382	193	72	247	160	12	0.7	6	14	3.0	8
Mean	1.6 B	303.1 A	152.3	51.1 B	180.8 A	116.0	10 A	0.2 B	5.0	14.3 A	3.9 B	9.1
	01 June 1995			*15 August 1995*			*01 June 1995*			*15 August 1995*		
Control	5	20	13 b	25 Bb‡	197 Aa‡	111	13	7	10	5	5	5
Peanut hay	16	101	57 ab	258 Aa‡	180 Aa‡	219	19	17	18	19	4	11
Woodchips	76	104	90 a	48 Ab‡	259 Aa‡	154	11	7	9	10	15	13
Mean	32.6 B	75.1 A	75.1	110.2	212.2	161.2	14.4	10.2	12.3	11.4 A	7.9 A	9.7
	08 May 1996			*07 August 1996*			*08 May 1996*			*07 August 1996*		
Control	3	24	14	49 Bb‡	212 Aab‡	131	11	5.5	8	12	10	11
Peanut hay	15	15	15	337 Aa‡	125 Bb‡	231	10	4.5	7	9	15	12
Woodchips	10	13	12	206 Aa‡	273 Aa‡	239	3	5.0	4	15	9	12
Mean	9.4 B	17.5 A	13.5	197.6	203.2	200.4	8.1	5.0	6.5	12.2	11.5	11.9

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different ($P \leq 0.05$), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at $0.05 \leq P \leq 0.10$.

Table 7.6. Effect of the organic amendment on gall and egg mass indices of *Meloidogyne arenaria* on 'Clemson Spineless' okra in microplot experiments.

Amendment	Gall index‡			Egg mass index‡		
	Low level	High level	Mean	Low level	High level	Mean
			1994			
Control	1.8†	4.3	2.7	.	.	.
Peanut hay	1.5	4.9	3.2	.	.	.
Woodchips	2.1	4.8	3.4	.	.	.
Mean	1.6 B	4.7 A	2.8	.	.	.
			1995			
Control	1.1 Bb	5.0 Aa	3.1	0.6 Bb	3.8 Aa	2.2
Peanut hay	3.9 Aa	4.9 Aa	4.4	2.6 Aa	3.3 Aa	2.9
Woodchips	2.8 Ba	5.0 Aa	3.9	2.2 Aa	3.3 Aa	2.7
Mean	2.6	5.0	3.7	1.8	3.4	2.5
			1996			
Control	2.7 Bb	4.3 Aa	3.5	1.7 Bb	2.8 Aa	2.3
Peanut hay	4.0 Aa	4.1 Aa	4.1	3.4 Aa	3.2 Aa	3.3
Woodchips	3.9 Aa	4.5 Aa	4.2	2.8 Aa	3.2 Aa	3.0
Mean	3.6	4.3	3.9	2.6	3.1	2.8

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different ($P \leq 0.05$), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Egg masses and galls rated on 0 to 5 scale, where 0 = 0 gall or egg mass per root system; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 galls or egg masses per root system.

arenaria (Tables 7.4, 7.5). In the following years, stubby-root nematodes reached similar levels in all treatments (Tables 7.4, 7.5).

Rye Cover Crop

The depth of straw due to the rye hay residues was affected by the amendment ($P \leq 0.01$) only after the first year (Table 7.7). However, nematode level had a significant impact on the depth of the straw ($P \leq 0.05$) on all dates of measurements (Table 7.7). For all measurements, the greatest depth of straw was recorded under high nematode level (Table 7.8). There was no significant difference between the two amendments, but in the first year, peanut hay and woodchip amendments resulted in the highest layer of straw ($P \leq 0.01$). The greatest weight of rye heads and stems were recorded under the high nematode level. Rye head weight was higher under peanut hay or woodchip amendments (Tables 7.7 and 7.9).

Soil Properties and Nutrients

Organic amendment affected organic matter in soil only in November 1995 and May 1996 ($P \leq 0.05$) (Table 7.10). The highest percentage of organic matter was registered under peanut hay amendment in May 1996 (Table 7.11). There was a significant ($P \leq 0.01$) interaction between amendment and nematode level for pH on August 1995 (Table 7.10). The interaction probably was due to dryness during this period, since for the remaining data, amendment did not affect soil pH (Table 7.10).

Table 7.7. Effect of the nematode (*Meloidogyne arenaria*) population level and organic amendment on hay produced by rye cover crop. Data shown are F-values from the analysis of variance.

Treatment	Depth of rye hay layer				
effect	June 1995	November 1995	June 1996	Rye head weight	Rye stem weight
Nematode level(A)	5.39*	5.65*	7.61*	8.13*	8.81**
Amendment(B)	6.05**	0.79 ns	2.23 ns	5.88*	3.32 ns
Interaction(AB)	0.21 ns	2.74 ns	0.63 ns	2.31 ns	3.66 @

*, ** Significant effects at P≤ 0.05 and P≤ 0.01, respectively; @ indicates significant at 0.05 ≤ P ≤ 0.10, ns= effect not significant at P≤0.10.

Table 7.8. Effect of nematode (*Meloidogyne arenaria*) population level and organic amendment on depth of rye hay layer.

Organic amendment	Depth of rye hay layer (cm)		
	Low	High	Mean
		June 1995	
Control	2.62	3.37	3.00 b
Peanut hay	4.50	5.94	5.20 a
Woodchips	3.00	4.50	3.70 ab
Mean	3.37 B	4.60 A	3.98
		November 1995	
Control	0.62	1.25	0.94
Peanut hay	1.00	0.87	0.94
Woodchips	0.50	1.00	0.75
Mean	0.71 B	1.04 A	0.85
		June 1996	
Control	1.25	1.75	1.50
Peanut hay	1.94	2.94	2.40
Woodchips	1.19	2.69	1.90
Mean	1.46 B	2.46 A	1.96

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

Table 7.9. Effect of nematode (*Meloidogyne arenaria*) population level and organic amendment on weight of stem and head of rye. September 1996.

Organic amendment	Head weight (g)			Stem weight (g)		
	Low	High	Mean	Low	High	Mean
Control	47	47	47 b	136 Aa	124 Aa‡	130
Peanut hay	62	89	76 a	154 Aa	226 Aa	190
Woodchips	32	69	51 ab	85 Ba	202 Aa	148
Mean	47 B	68 A	58	125	184	155

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at 0.05≤P≤0.10.

Table 7.10. Effect of organic amendment and nematode (*Meloidogyne arenaria*) population level on soil organic matter and pH. Data shown are F-values from the analysis of variance.

Treatment effect	Organic matter (%)	pH
	September 1994	
Nematode level (A)	1.42 ns	0.32 ns
Organic amendment (B)	0.80 ns	5.68*
Interaction (A x B)	1.05 ns	1.79 ns
	May 1995	
Nematode level (A)	6.49*	0.11 ns
Organic amendment (B)	2.22 ns	0.44 ns
Interaction (A x B)	0.28 ns	1.50 ns
	August 1995	
Nematode level (A)	0.01 ns	46.09**
Organic amendment (B)	2.33 ns	11.75**
Interaction (A x B)	2.49 ns	44.45**
	November 1995	
Nematode level (A)	5.47*	3.27 ns
Organic amendment (B)	3.62*	3.15 ns
Interaction (A x B)	0.57 ns	2.72 ns
	May 1996	
Nematode level (A)	0.13 ns	1.13 ns
Organic amendment (B)	3.84*	1.25 ns
Interaction (A x B)	0.23 ns	1.36 ns

*, ** Significant effects at P≤ 0.05 and P≤ 0.01, respectively; ns= effect not significant at P≤0.05.

Table 7.11. Effect of nematode *(Meloidogyne arenaria)* population level and organic amendment on soil organic matter and pH in microplot experiments.

Treatment	Organic matter (%)			pH		
effect	Low level	High level	Mean	Low level	High level	Mean
			September 1994			
Control	0.65	1.03	0.84	6.85	7.02	6.94 ab
Peanut hay	0.98	1.00	0.99	6.90	6.75	6.82 b
Woodchips	1.00	1.01	1.00	7.07	7.17	7.12 a
Mean	0.87	1.02	0.94	6.94	6.98	6.96
			May 1995			
Control	1.17	0.84	0.98	6.50	6.65	6.57
Peanut hay	1.45	1.03	1.24	6.72	6.37	6.55
Woodchips	1.08	0.87	0.98	6.65	6.72	6.69
Mean	1.21 A	0.91 B	1.06	6.62	6.58	6.60
			August 1995			
Control	1.10	1.16	1.13	6.35 Bb	6.52 Ab	6.44
Peanut hay	1.20	1.30	1.25	6.50 Aa	6.40 Bc	6.45
Woodchips	1.22	1.07	1.14	6.40 Bb	6.65 Aa	6.52
Mean	1.17	1.18	1.17	6.42	6.52	6.47
			November 1995			
Control	0.86	0.97	0.92 b‡	7.15	7.17	7.16
Peanut hay	1.00	1.33	1.16 a‡	7.17	6.85	7.01
Woodchips	1.08	1.24	1.16 a‡	7.22	7.17	7.20
Mean	0.98 B	1.18 A	1.08	7.18	7.07	7.12
			May 1996			
Control	1.28	1.04	1.08 b	7.15	7.32	7.24
Peanut hay	1.31	1.29	1.30 a	7.15	7.05	7.10
Woodchips	1.11	1.14	1.13 ab	7.02	7.20	7.11
Mean	1.18	1.16	1.17	7.11	7.19	7.15

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different ($P \leq 0.05$), according Tukey's test. Capital letters stand for comparisons between main effect means in columns or in rows.

‡ Significant at $0.05 \leq P \leq 0.10$.

Among macronutrients, N, K, and Ca were the most affected by amendment, P and Mg the least (Table 7.12). For all collection dates, N was higher under peanut hay and woodchip amendments (Table 7.13). For K, there was no significant difference between amendment and control treatment in the first year. However, on the second collection date, soil K content from the peanut hay amendment treatment was almost double that of the control treatment (Table 7.13). In general, levels of N and K tended to be higher in soil of plots with the high root-knot nematode levels (Table 7.13). There were no interactions between amendment and nematode level for Ca and Mg except in August 1995 (Table 7.12). There is a possibility that the dryness during this period of collecting data may have affected this interaction. There was not much variability in soil P. The average level of P in soil from September 1994 to May 1996 was 64.6 ppm (sd= 3.6).

Organic amendment affected Mn soil level (Table 7.14). The highest values were recorded under woodchip amendment treatment (Table 7.15). In general, micronutrients were not affected by treatments (Table 7.14). An exception was noted with data from soil sampled in August 1995, where all micronutrients analyzed were affected by both nematode and organic amendment (Table 7.14). These results were similar to those obtained for the macronutrients. Again, there is a possibility that dryness may have affected the August 1995 results.

Table 7.12. Effect of organic amendment and nematode (*Meloidogyne arenaria*) population level on soil macronutrients. Data shown are F-values from the analysis of variance.

Treatment effect	N	P	K	Ca	Mg
			September 1994		
Nematode level (A)	8.07*	0.40 ns	13.12**	1.06 ns	2.77 ns
Organic amendment (B)	9.97**	1.62 ns	2.86 ns	8.73*	1.18 ns
Interaction (A x B)	0.88 ns	0.34 ns	0.35 ns	0.96 ns	2.78 @
			May 1995		
Nematode level (A)	9.82**	0.37 ns	9.96**	2.08 ns	2.98 ns
Organic amendment (B)	7.57**	0.43 ns	23.68**	0.11 ns	1.99 ns
Interaction (A x B)	0.15 ns	0.25 ns	0.54 ns	0.65 ns	1.54 ns
			August 1995		
Nematode level (A)	18.12**	1.18 ns	135.50**	29.27**	4.56*
Organic amendment (B)	12.96**	0.27 ns	765.31**	6.23**	7.81**
Interaction (A x B)	3.98*	3.38 ns	9.78**	24.95**	14.12**
			November 1995		
Nematode level (A)	3.25 ns	0.28 ns	5.71*	0.22 ns	0.18 ns
Organic amendment (B)	4.28*	1.73 ns	8.45**	5.44*	3.81 ns
Interaction (A x B)	1.17 ns	0.44 ns	0.84 ns	0.12 ns	0.56 ns
			May 1996		
Nematode level (A)	1.51 ns	2.48 ns	0.12 ns	3.88 ns	0.25 ns
Organic amendment (B)	6.41**	2.51 ns	6.56**	1.63 ns	2.65 ns
Interaction (A x B)	0.50 ns	0.30 ns	0.05 ns	0.69 ns	1.78 ns

*, ** Significant effects at $P \leq 0.05$ and $P \leq 0.01$, respectively; @ indicates significant at $0.05 \leq P \leq 0.10$, ns= effect not significant at $P \leq 0.10$.

Table 7.13. Effect of nematode *(Meloidogyne arenaria)* population level and organic amendment on soil macronutrients in microplot experiments.

Treatment effect	N (%)			K (ppm)			Ca (ppm)			Mg (ppm)		
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
						September 1994						
Control	0.0207	0.0200	0.0204 b	104	132	118	453	530	492 b	40.6	33.3	37.0
Peanut hay	0.0252	0.0230	0.0241 a	127	174	151	502	477	490 b	43.7	34.9	39.3
Woodchips	0.0245	0.0215	0.0230 a	113	160	137	605	647	626 a	39.5	43.5	41.5
Mean	0.0215 B	0.0235 A	0.0225	115 B	155 A	135	520	551	536	41.3	37.2	39.2
						May 1995						
Control	0.0185	0.0215	0.0199 b	108	144	126 b	414	520	467	42.3	44.5	43.4
Peanut hay	0.0237	0.0287	0.0262 a	229	309	269 a	482	486	484	58.1	45.1	51.6
Woodchips	0.0217	0.0262	0.0240 a	139	190	165 b	462	511	487	54.1	46.3	50.2
Mean	0.0215 B	0.0255 A	0.0234	159 B	214 A	186	453	506	479	51.5	45.3	48.4
						August 1995						
Control	0.0167 Aa	0.0182 Ab	0.0175	92 Bc	120 Ac	106	359 Bb	448 Aab	403	35.0 Ab	34.4 Ab	34.7
Peanut hay	0.0185 Ba	0.0252 Aa	0.0219	274 Ba	351 Aa	313	451 Aa	408 Ab	430	47.7 Aa	34.3 Bb	41.0
Woodchips	0.0162 Aa	0.0185 Ab	0.0174	132 Bb	185 Ab	159	390 Bb	498 Aa	444	38.0 Ab	42.8 Aa	40.4
Mean	0.0172	0.0207	0.0189	166	218	192	400	451	426	40.2	37.2	38.7
						November 1995						
Control	0.0177	0.0205	0.0191 b	87	91	89 b	540	577	558 b	34.3	35.9	35.1
Peanut hay	0.0215	0.0262	0.0239 a	107	131	119 a	607	631	619 ab	44.3	40.7	42.5
Woodchips	0.0222	0.0220	0.0221 ab	104	119	111 a	716	709	712 a	45.8	53.3	49.6
Mean	0.0205	0.0217	0.0217	99 B	114 A	106	621	639	630	41.5	43.3	42.4
						May 1996						
Control	0.0172	0.0177	0.0175 b	98	97	98 b	536	621	578	34.1	34.9	34.5
Peanut hay	0.0217	0.0255	0.0236 a	159	167	163 a	621	642	632	42.9	36.0	39.4
Woodchips	0.0190	0.0200	0.0195 ab	128	136	132 ab	600	742	671	40.1	52.5	46.3
Mean	0.0193	0.0211	0.0211	128	133	131	586	668	627	39.0	41.1	40.1

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at 0.05≤P≤0.10.

Table 7.14. Effect of organic amendment and nematode (*Meloidogyne arenaria*) population level on soil micronutrients. Data shown are F-values from the analysis of variance.

Treatment effect	Zn	Cu	Na	Fe	Mn
			September 1994		
Nematode level (B)	2.23 ns	1.30 ns	0.01 ns	0.00 ns	0.68 ns
Organic amendment (A)	2.53 ns	1.24 ns	1.62 ns	1.51 ns	3.55*
Interaction (A x B)	0.78 ns	0.73 ns	0.95 ns	0.74 ns	0.42 ns
			May 1995		
Nematode level (B)	0.88 ns	1.75 ns	0.05 ns	0.05 ns	2.72 ns
Organic amendment (A)	1.05 ns	2.90 ns	1.73 ns	0.70 ns	0.58 ns
Interaction (A x B)	0.14 ns	0.20 ns	2.04 ns	0.68 ns	0.09 ns
			August 1995		
Nematode level (B)	70.68**	1.60 ns	0.03 ns	5.58*	0.05 ns
Organic amendment (A)	7.01**	63.64**	0.95 ns	26.35**	8.89**
Interaction (A x B)	28.82**	53.68**	8.59**	12.78**	0.37 ns
			November 1995		
Nematode level (B)	0.23 ns	0.00 ns	3.68 ns	0.02 ns	0.03 ns
Organic amendment (A)	2.92 ns	0.87 ns	1.61 ns	0.48 ns	7.89**
Interaction (A x B)	0.51 ns	0.11 ns	0.02 ns	0.34 ns	0.20 ns
			May 1996		
Nematode level (B)	3.10 ns	0.68 ns	0.81 ns	2.14 ns	1.01 ns
Organic amendment (A)	2.55 ns	0.94 ns	1.50 ns	0.51 ns	5.23*
Interaction (A x B)	1.31 ns	0.57 ns	1.09 ns	0.37 ns	0.77 ns

*, ** Significant effects at P≤ 0.05 and P≤ 0.01, respectively; ns= effect not significant at P≤0.05.

Table 7.15. Effect of the nematode (*Meloidogyne arenaria*) population level and organic amendment in soil mineral analysis in microplots experiments.

Treatment effect	Zn			Cu			Fe			Mn		
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
						September 1994						
Control	2.43	3.04	2.7	0.52	0.52	0.5	11.4	11.3	11	4.08	4.50	4.3 ab‡
Peanut hay	2.62	2.59	2.6	0.53	0.39	0.5	10.7	10.7	11	4.09	4.26	4.2 b‡
Woodchips	3.01	4.15	3.6	0.57	0.54	0.5	12.9	12.0	12	4.86	4.80	4.8 a‡
Mean	2.7	3.3	3.0	0.5	0.5	0.5	12	12	12	4.3	4.5	4.4
						May 1995						
Control	2.23	2.84	2.5	0.74	0.58	0.7 a‡	11.3	11.2	11	3.50	4.04	3.8
Peanut hay	3.03	3.21	3.1	0.47	0.42	0.4 b‡	10.6	11.3	11	3.94	4.23	4.1
Woodchips	2.94	3.17	3.0	0.61	0.53	0.6 a‡	12.3	11.3	12	3.79	4.16	4.0
Mean	2.7	3.1	2.9	0.6	0.5	0.6	11	11	11	3.7	4.1	3.9
						August 1995						
Control	2.88 Bb	3.71 Ab	3.3	0.58 Bc	0.79 Aa	0.7	13.8 Ab	14.3 Aa	14	6.40 Aa	6.20 Ab	6.3
Peanut hay	3.77 Aa	3.66 Ab	3.7	0.66 Ab	0.58 Bb	0.6	13.9 Ab	14.7 Aa	14	6.90 Aa	7.00 Aa	6.9
Woodchips	2.71 Bb	4.30 Aa	3.5	0.84 Aa	0.76 Ba	0.8	15.8 Aa	14.4 Ba	15	6.30 Aa	6.30 Aa	6.3
Mean	3.1	3.9	3.5	0.7	0.7	0.7	14	14	14	6.5	6.5	6.5
						November 1995						
Control	3.37	2.96	3.2	0.60	0.59	0.6	11.4	11.1	11	4.50	4.40	4.4 b
Peanut hay	3.52	3.70	3.6	0.51	0.48	0.5	10.2	11.1	11	4.80	5.00	4.9 ab
Woodchips	4.41	5.57	5.0	0.54	0.58	0. 6	11.6	11.3	11	5.40	5.40	5.4 a
Mean	3.8	4.1	3.9	0.5	0.5	0.5	11	11	11	4.9	4.9	4.92
						May1996						
Control	3.34	3.77	3.55	0.73	0.63	0.7	11.6	10.8	11	4.60	4.80	4.7 b
Peanut hay	3.90	4.23	4.06	0.66	0.52	0.6	11.7	11.2	11	5.30	5.20	5.2 ab
Woodchips	3.97	6.46	5.21	0.69	0.74	0.7	13.1	11.1	12	5.40	6.10	5.7 a
Mean	3.74	4.82	4.28	0.7	0.6	0.7	12.1	11	11	5	5	5

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Indicates significant at 0.05≤P≤0.10

Plant Nutrients

The highest N in leaf tissue was detected in plants with high nematode level (Table 7.16, 7.17). In general, amendment affected macronutrients levels in leaf blades, but not in petioles, although effects on the different macronutrients varied (Table 7.16, 7.17).

Among micronutrients in leaf blades, only Mn was affected ($P \leq 0.01$) by both nematode level and amendment (Table 7.18). Micronutrient responses were detected more readily in petioles than in leaf blades (Table 7.18). Levels of Zn, Cu, and Fe, in petiole were higher in the high nematode treatment, and in the case of Fe the highest level resulted in plots with high nematode treatment amended with woodchips (Table 7.19).

Results of mineral analyses differed when nutrients were extracted from leaf blades or petioles (Tables 7.16, and 7.18). Since the weight of plant tissue (leaf and petiole) sampled for the nutrient analyses were not affected by inoculum or amendment (data not shown), the variation observed in the nutrient concentration was attributed to the plant itself. Average fresh weight of leaf blade was 0.99 g per plant, and of petiole was 0.24 g. However, conclusions about relationships between nutrient and disease or nematode level are limited in this case. Repeated analyses would be needed at different plant stages, because nutrient uptake may vary among plant parts and plant growth stage.

Discussion

Okra growth was affected by the nematode level. These effects were expected since plots were set up with low or high root-knot nematode levels. Therefore, the plant

Table 7.16. Effect of organic amendment and nematode (*Meloidogyne arenaria*) population level on plant macronutrients in microplot experiment. Data shown are F-values from the analysis of variance.

Treatment effect	N	P	K	Ca	Mg
			Leaf blade		
Nematode level (A)	45.00**	2.38 ns	0.01 ns	4.36 @	9.51**
Organic amendment (B)	1.31 ns	11.49**	8.32**	6.80**	2.49 ns
Interaction (A x B)	4.47*	2.46 ns	0.32 ns	1.25 ns	0.82 ns
			Petiole		
Nematode level (A)	21.92**	11.06**	8.04*	0 ns	6.59*
Organic amendment (B)	3.53 @)	2.37 ns	0.93 ns	2.32 ns	0.32 ns
Interaction (A x B)	0.58 ns	0.65 ns	3.33 @	0.03 ns	2.12 ns

*, ** Indicate significant main effect or interaction at P ≤0.05 and P≤0.01, respectively, @ indicates significant at 0.05 ≤ P ≤ 0.10, ns= effect not significant at P≤0.10.

Table 7.17. Effect of nematode (*Meloidogyne arenaria*) population level and organic amendment in plant macronutrients in microplots experiments.

Treatment effect	N (%)			P (ppm)			K (ppm)			Ca (ppm)			Mg (ppm)		
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
	Leaf blade														
Control	1.59 Ba	2.61 Aa	2.1	6,675	7,300	6,987 a	6,565	5,797	6,181 b	59,000	50,975	55,237 a	8,465	6,250	7,357
Peanut hay	1.75 Aa	2.03 Ab	1.9	6,275	6,225	6,250 b	9,355	10.052	9,704 a	48,425	42,925	45.675 b	6,297	5,460	5,879
Woodchips	1.56 Ba	2.40 Aab	2.0	6,354	6,400	6,377 b	6,290	6,640	6,465 b	47,619	47,625	47,622 b	7,242	4,495	5,868
Mean	1.64	2.34	2.0	6,434	6,641	6, 538	7.403	7,497	7.450	51,848 A‡	47,175 B‡	49,511	7,335 A	5,402 B	6,368
	Petiole														
Control	0.69	1.07	0.88 a‡	6,422	9,490	7,956	9,398 Aa‡	4,861 Aa‡	7,129	30,805	29,978	30,391	12,387	9,246	10,816
Peanut hay	0.55	0.77	0.66 b‡	6,259	7,493	6,876	7,511 Aa‡	8,354 Aa‡	7,932	26,365	27,065	26,715	9,954	10,226	10,090
Woodchips	0.51	0.89	0.7 ab‡	7,480	9,763	8,621	11,741 Aa‡	6.148 Ba‡	8,945	34,024	34,186	34,105	12,670	8,962	10,816
Mean	0.58 B	0.91 A	0.75	6,720 B	8,915 A	7,818	9,550	6,454	8,002	30,398	30,410	30,404	11,670 A	9,478 B	10,574

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at 0.05≤P≤0.10.

Table 7. 18. Effect of organic amendment and nematode (*Meloidogyne arenaria*) population level on plant micronutrients. Data shown are F-values from the analysis of variance.

Treatment effect	Zn	Cu	Fe	Mn
			Leaf blade	
Nematode level (A)	0.84 ns	1.43 ns	5.64*	35.39**
Organic amendment (B)	2.86 @	1.57 ns	0.13 ns	9.84**
Interaction (A x B)	0.06 ns	2.22 ns	0.12 ns	0.36 ns
			Petiole	
Nematode level (A)	17.34**	4.89*	12.29**	0.34 ns
Organic amendment (B)	3.75*	3.17 @	8.26**	2.01 ns
Interaction (A x B)	0.91 ns	0.12 ns	4.83*	2.37 ns

*, ** Indicate significant main effect or interaction at P ≤0.05 and P≤0.01, respectively, @ indicates significance at 0.05 ≤ P ≤ 0.10, ns= effect not significant at P≤0.10.

Table 7.19. Effect of nematode (*Meloidogyne arenaria*) population level and organic amendment in plant mineral analysis in microplot experiment.

Treatment effect	Zn			Cu			Fe			Mn		
	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean	Low level	High level	Mean
	Leaf blade											
Control	103	98	100 a‡	14	16	15	120	165	142	182	94	138 b
Peanut hay	80	67	73 b‡	15	10	13	115	172	144	232	165	199 a
Woodchips	95	87	91 ab‡	14	13	13	115	150	133	175	110	143 b
Mean	93 A	84 B	88	14.4	13	13.7	116.7 B	162.5 A	139.6	197 A	123 B	160
	Petiole											
Control	97	138	118 b‡	7.2	12.5	9.9 ab‡	55 Aa	155 Ab	105	51	29	40
Peanut hay	100	134	117 b‡	6.7	9.7	8.2 b‡	43 Aa	100 Ab	71	43	45	44
Woodchips	116	187	152 a‡	11.7	16.2	14.0 a‡	109 Ba	581 Aa	345	50	59	54
Mean	105 B	153 A	129	8.6 B	12.8 A	10.7	69	279	174	48	44	46

† Data are means of four replications. Means followed by the same small letters in columns are not significantly different (P≤0.05), according to Tukey's test. Capital letters stand for comparisons between means in rows.

‡ Significant at 0.05≤P≤0.10.

data confirmed these expected effects. It is interesting to observe that the highest response was recorded under peanut hay amendment, especially in the first year. One of the reasons is the fact that fertilizer was applied only in the first year. In addition, analysis of the peanut hay revealed high levels of mineral nutrients, which might be released faster from peanut hay than from woodchips or rye hay, because peanut hay had the lowest C/N ratio. Although nutrients from amendments and fertilizer affected plant growth, application of fertilizers as a means of managing nematodes is still controversial. For instance, Rodríguez-Kábana (1986) mentioned that fertilizer rates for nematode management must be higher than rates necessary for crop fertilization. Furthermore, damage caused by nematodes can be overcome to various degrees by application of fertilizer (Norton, 1978). It has been reported that moderate fertilization resulted in increase of *Pratylenchus* spp., however no N-P-K application in corn resulted in higher levels of *Helicotylenchus penetrans* (Norton, 1978). In another study, Huber (1980) mentioned that NH_4 and Mg required for plant growth are reported to increase infection of *M. incognita* in lima bean and cucumber. On the other hand, NO_3 or P are reported to reduce the severity, while K effect will depend on the environment. This same author mentioned that K has been reported to decrease *M. javanica* infection but, at the same time promoted increase of *Tylenchus semipenetrans*.

In our study, nutrient levels were higher in the first year, but nutrient levels could not be associated with nematode density. Although a specific nutrient may promote some disease while reducing others, effect on disease is still dependent on environmental conditions (Huber, 1980).

Recently, a study of relationships between nematode density and nutrient uptake indicated few consistent correlations between nematode densities and level of nutrient elements in leaves (McSorley and Gallaher, 1992). Increase of nematode population also was dependent upon the crop grown. These authors mentioned that effects of nematode population on nutrient uptake are more easily observed when plants are under yield-limiting stress from nematodes.

In our experiment, supplementary fertilizer was applied only in the first year, thus most soil nutrients were added through the decomposition of amendments. However, levels of N varied consistently with the soil amendment; in the non-amendment control treatment, N level was lowest and tended to decrease over time (average of 0.0204 to 0.0175 %). In the other amendment treatments, the N level was higher compared to the non-amendment control (average of 0.0235 to 0.0215 over time). In addition, among peanut hay, woodchip, and rye hay, the peanut hay amendment had the lowest C/N ratio, and woodchip the highest. Thus, higher levels of mineral elements were generally detected after amendment with peanut hay because this amendment decomposed faster than woodchips or rye hay.

It was remarkable that rye yields as well as rye hay depths were greater in plots with high root-knot nematode levels. Consistently, higher levels of soil nutrients were available in these plots as well. Of course, plant damage and root-galling were greater in these plots, and so it is likely that okra roots damaged by the root-knot nematodes were impaired in their ability to uptake nutrients. This hypothesis is also supported by the fact that N and K in soil were higher in plots with high root-knot nematode levels, and thus rye, a relatively weak host of root-knot nematodes (McSorley, 1994), had more nutrients

available in these plots. Rye growth was best in plots with the peanut amendment, where available K was particularly high.

On all sample dates in the first year, *Paratrichodorus minor* counts were greater in plots with low root-knot nematode levels, possibly because there was competition from root-knot nematodes. According to Christie (1959), *Paratrichodorus* spp. feed on root tips. Thus, in plots with high levels of root-knot nematodes, there were probably less feeding sites for *P. minor* due to the presence of galls on the root system. Since nematode infection in plots with low levels of root-knot nematode had a tendency to increase over time, the levels of *P. minor* tended to stabilize.

There is not enough evidence to make conclusions about effects of nematode infection or population density based on mineral analysis of okra leaf blades and petioles. However, it is important to understand that the mineral nutrients vary in different plant tissue and over time. Thus, to generate conclusions about nematode population levels, the optimum level of each element should be known for each specific case and analyses should be repeated to achieve consistency of the data and to make statistical comparisons.

Okra yields showed decreasing trends in the second and third year of the experiment. One reason might be the fact that microplots were cultivated in the same area, and did not receive supplemental fertilizer to compensate for nutrients that were no longer available for plant uptake. Secondly, although microplots were over-planted with 7 okra seedlings each, this number of plants utilized was still not adequate for realistic assessment of yields.

CHAPTER 8
CONCLUSIONS

Eight different organic amendments were used to manage root-knot nematode (*Meloidogyne incognita* and *M. arenaria*) in greenhouse and microplot experiments. Those organic amendments were tested on a highly susceptible 'Clemson Spineless' okra. Preliminary experiments revealed that two-week-old okra seedlings were best for building up root-knot nematode populations and studying nematode population dynamics under greenhouse conditions. Positive effects of organic amendments on plant growth were more evident when amendments were placed on soil surface as a mulch, rather than incorporated. In addition, irrigation frequency had a profound effect on mulched plants when compared to unmulched plants.

Plant growth responses and nematode suppression varied with different kinds of amendments. Normally, greater responses in plant growth and nematode suppression were registered under amendments with low C/N ratios (castor, collard, velvetbean, and zinnia), than under amendments with high C/N ratios (sesame and sorghum). Differences in responses occurred when amendments were applied fresh or dry, however these differences disappeared when a fresh weights corresponding to equivalent dry weights were used. It cannot be generalized that amendments with low C/N ratios will always suppress nematodes and improve plant growth, if conditions of soil moisture, temperature, plant host, and nematode are not kept constant. Time for the amendment

decomposition had a great effect on plant responses, however nematode population density was also an important factor affecting plant responses.

The rate of the amendment applied had a profound effect on plant growth and nematode population level. However, at a certain point, an amendment rate might be toxic to the plant, before having effect on nematode population. In most cases, the relationships between plant responses and amendment rate or between nematode level and amendment rate were quadratic, suggesting an optimum rate at which the amendment was most effective. For instance, a rate of 5 g of dry velvetbean or castor amendment per 560 cm^3 of soil gave the best suppression of nematodes.

Among amendments, velvetbean was more effective in suppressing nematodes and improving plant growth when compared to a similar rate of castor amendment. Although, C/N ratio is very important in determining the use of an amendment the amount to be utilized must also be considered for economic reasons. Because velvetbean is more dense than castor less amount of fresh velvetbean amendment than castor will be required.

Results from microplot experiments revealed high soil nutrient levels in microplots with high levels of root-knot nematodes, thus galled roots impaired plant nutrient uptake. However, results of nutrient analysis in the plant depend on plant growth stage because nutrient concentrations vary in plant tissue. Thus, correlation between nutrient levels and nematode population levels should account for this factor when interpreting suppression of nematodes with organic amendments. The presence of other plant-parasitic nematodes may also interfere with plant response. A cover crop, such as

the rye used in the microplot experiment, had a profound effect on nematode responses, because of its different host status to root-knot and stubby-root nematodes. Thus, field studies should account for the presence of other plant-parasitic nematodes when interpreting results.

Although, nematode suppression and improvement of plant growth through the use of amendments have been demonstrated, it is important to mention that even the most effective chemical nematicide is not 100 % guaranteed to eliminate all nematodes. Thus, the remaining nematode population will be able to build up rapidly in the presence of a suitable host (Singh and Sitaramaiah, 1994). An integrated management approach, by using an organic amendment with low C/N ratio and a non-host crop, could be adopted for the effective use of the amendment for suppression of the nematode and improvement of plant growth.

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BIOGRAPHICAL SKETCH

Cecília Helena Silvino Prata Ritzinger was born in Viçosa, Minas Gerais, Brazil. She attended elementary, middle, and high school in Vitória, Espírito Santo. In 1977, she entered the Federal University of Viçosa, Minas Gerais, from which she received a Bachelor of Science degree in agronomy in 1980. After graduation, she received training in plant disease diagnosis at EMCAPA (a state agricultural research agency), Vitória, Espírito Santo, and in irrigation at the University of Lavras, Minas Gerais, and worked in the Provárseas program in the south of Bahia. She participated in the Probor II, in Altamira, Para. She taught in an elementary school in Vitória, Espirito Santo. In 1982, she was awarded a scholarship from EMBRAPA (a Brazilian agricultural research agency) for continuing her education at the Federal University of Rio Grande do Sul, pursuing a Master of Science degree in Fitossanidade. She obtained this degree in 1984. Her research dealt with diseases of yellow passion fruit (*Passiflora edulis* var. *flavicarpa*). In 1985, she was approved, through selection, to work in EMBRAPA as a plant pathology researcher, in the state of Acre, Brazil. Most of her work there was related to identification of plant diseases. She was responsible for collecting and maintaining a germplasm collection of cassava for evaluation and characterization, and management of web-blight disease by using organic amendments in black beans. In 1994, she was accepted by the Entomology and Nematology Department as a graduate

student in a Ph.D. program with concentration in nematology with a minor in plant pathology. She was also awarded a scholarship from EMBRAPA for this purpose. After the completion of the requirements of the Ph.D. program, she intends to continue her professional career in Brazil and spend weekends with her family.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert McSorley, Chair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Gary W. Simone
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Raymond N. Gallaher
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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